Modelling seed germination and seedling survival of *Eucalyptus delegatensis* R. T. Baker to facilitate optimal reafforestation.

by

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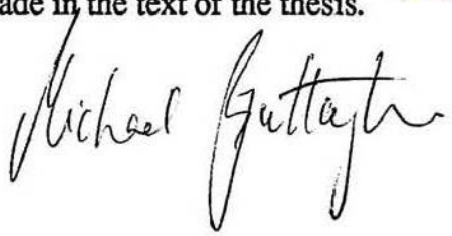
This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and to the best of my knowledge, and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.



Abstract

Land managers increasingly are being involved in making quantitative evaluation of management options. Forests, however, are complex biological systems and predictions require the synthesis of many processes. Traditional approaches to evaluating options have been to replicate experiments in time and space. Not all questions are amenable to such approaches, and even where they are, inferences may be of only limited application. In an increasingly complex decision making context, land managers will require access to more sophisticated techniques. This thesis illustrates the collection of basic data, its synthesis into a physiological model, and its use as a tool to address a typically complex management question - the time at which *Eucalyptus delegatensis* R.T.Baker seed should be sown in forest regeneration operations. The species is widely exploited for commercial forestry in South-Eastern Australia and its germination physiology is moderately complex, providing an appropriate test for the usefulness of the modelling approach.

The germination response of seedlots from five provenances to temperature, stratification, soil matric potential and interrupted imbibition was examined. The species was found to have a distinct temperature optimum between 15 and 20°C, and a minimum temperature for germination of approximately 2°C. Short periods of exposure to high temperatures did not substantially affect germination performance. Stratification greatly increased the range of temperatures over which a high proportion of the seed germinated. Increases in the rate of germination with stratification are related to accrued thermal time during stratification. Pre-imbibing seeds at water potentials down to -2 MPa increased the rate of germination. However, no advantage was found after pre-imibibing at lesser soil water matric potentials. This increased germination rate was associated with a shortening of the time to commencement of germination and more synchronous germination. Germination rate and germination capacity were impeded by soil matric potentials below -0.01 MPa, and germination was totally inhibited by soil matric potentials below -0.5 MPa. Soil matric potential and temperature interacted in their effects on germination capacity, and seeds germinating at near optimum temperatures were less sensitive to soil moisture stress. Seeds survived dehydration within sixty hours of the commencement of imbibition, but were increasingly affected by dehydration thereafter. The rate of imbibition was influenced by the ambient temperature and solution water potential. At modest levels of water stress imbibition was not impeded and the observed reduction in germination capacity was probably due to the inhibition of

growth related processes. Differences in germination response were detected between the seedlots and these could be related to their geographic origin.

The proportion of variability in seed and germination traits attributable to inter- and intra-site components varied between traits examined. The germination rate of seed was not significantly different between trees within a site, or between trees from different sites. Variation in seed size and the proportion of dormant seed in seed samples was mainly affected by site effects. The sensitivity of seed samples to the water stress levels applied also varied substantially between sites but additionally the seed from the drier site exhibited a highly significant between-tree variability. It was concluded that the proportion of variation in seed and germination characteristics attributable to between-, and to within-site effects, could be partly related to the scale at which selective forces were presumed to operate. Nevertheless, a substantial amount of variation in response existed within the seed collected from the one tree.

The role of age and microtopographical variation in enabling seedlings to withstand frost and drought was explored in glasshouse studies. The frost resistance of *E. delegatensis* was found to vary with seedling age over the first six months of development. Much of this variation was found to be a result of the differing sensitivity of leaves originating from different leaf nodes, although older leaves from the same node may have been more frost resistant than recently expanded leaves. Newly emergent seedlings appeared to be the most susceptible stage of the tree's lifecycle to death by frost.

Small scale variation in soil conditions, at the scale of tens of centimetres, markedly affected the germination and establishment of seeds and seedlings under moisture limiting conditions. Microsites that afforded protection, and probably resulted in increased humidity, caused a marked increase in germination number and rate. The mean survival time was significantly higher on these protected microsites than on less protected microsites, or on microsites that restricted root penetration. The importance of this variability in microtopography was strongly influenced by season and the level of environmental stress, and was diminished as seedlings aged. Due to the different requirements for seed germination and seedling growth, a favourable microsite for germination was not necessarily a favourable site for seedling survival. A comparison of seed and seedling responses to water stress indicated that for *E. delegatensis*, at least, selection due to microsite differences at the time of germination may not affect the developmental characteristics of the seedlings.

At two geographically close sites that differed significantly in climatic profile, seed of *E. delegatensis* and *Eucalyptus amygdalina* Labill., a species that frequently replaces *E. delegatensis* on drier sites, was sown at twelve times of the year. Regular censuses of seedlings were conducted. The pattern of survival of over twenty thousand seedlings, comprising one thousand two hundred identified cohorts was followed. The influences of weather, seed harvesting, site preparation, time of emergence and time of sowing on emergence, growth and survival were examined. By modelling temperature and soil moisture it was found that germination in the field was influenced strongly by ambient temperature and soil moisture and that the commencement of germination flushes in spring and autumn were well correlated to threshold values of soil moisture and air temperature predicted from laboratory studies. A model of seasonal patterns of seedling mortality was developed and concluded to be highly age dependant. Although age dependant mortality rate was relatively constant at a given site between seasons and years, with each season containing its own compliment of hazards, it was necessary to make allowance for stochastic events, such as severe frosts and drought, to satisfactorily model survivorship.

A mathematical model of germination was developed for *E. delegatensis* based on the physiology of underlying processes. The accuracy of this model in predicting the time course of germination under conditions of fluctuating temperature and moisture was examined. This model was used to examine the results from the field trial. In combination with a mortality function derived from field observation, this germination model was used to make recommendations on the optimum times of sowing for the east coast of Tasmania, to explore the importance of 'safe sites' for germination, and to investigate the implications of different seedlot dormancy responses on reproductive success.

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Introduction

Chapter 1: Introduction

1.1 Preamble

Despite inherent variability, seeds respond predictably to environmental stimuli. This provides the basis for the predictive modelling of seed germination. Field emergence is, however, influenced by such phenomena as seed predation, senescence and spatial heterogeneity in soil conditions. The aims of this research are, therefore, to collect sufficient information on the physiological response of *Eucalyptus delegatensis* R.T. Baker seed to environmental stimuli, identify factors affecting the rate of seed removal from the ground seed store, and to model soil temperature and soil moisture so as to allow accurate prediction of field emergence. Questions of inter- and intra-provenance variability in seed germination characteristics, and how these interact with environmental graininess, will be examined to indicate the robustness of predictions. By examining experimentally the tolerance of seedlings at different ontogenetic stages to frost and drought, and the comparative growth rate and mean survival time of seedlings emerging at different times of the year this research aims to make recommendations on sowing practices to be used in reafforestation programs.

1.2 General Introduction

It is almost axiomatic to say that forest ecosystems are complex and consequently there is frequently considerable uncertainty about the consequence of disturbance. In some systems, only slight variations in post-disturbance conditions lead to substantially different community composition (e.g., Keenan and Candy 1983). Therefore, any information that reduces this uncertainty, and provides a quantitative evaluation of the state of the system over time, is of great value to the management of the system. Land managers, and in particular silviculturists, have, however, been supplied with little information upon which to base such evaluations (Biwas 1975; Landsberg 1986).

Most research into forestry problems continues to be essentially empirical (Landsberg 1986). This purely empirical approach has been successful in addressing a number of key forestry-management issues in which variation in weather conditions between years has not played a significant role in determining

the outcome. In particular, it has been successful in the comparative evaluation of felling regimes for the regeneration of different wet forest types (e.g. Gilbert 1959, 1960; Cunningham 1960; Florence and Crocker 1962). Not all problems, particularly management problems in forest types or locations that experience substantial between year variability in weather conditions, are as amenable to solution by empirical methods. In many instances the potential variation in environments and experimental conditions means that extrapolation of results from experimentation, without taking into account the inter-relationships between physiology and physical factors that caused the observed response, is imprudent (Landsberg 1986). Seldom is detailed environmental information collected when forestry field experiments are conducted. Furthermore, sufficient spatial and temporal replication to reduce the influence of seasonal or site effects on the outcome of experiments is usually beyond the scope of available resources. These problems are apparent in the case of determining the most appropriate time of year to sow eucalypt seed in reafforestation activities.

The successful regeneration of an area by direct seeding requires a coincidence in time and space of viable seed, a receptive seedbed and a period of favourable climatic and biotic conditions for germination and subsequent establishment. The silviculturist seeking to facilitate regeneration aims to maximise the coincidence of these factors. The time seed is sown impinges on each of these factors and, perhaps second only to the quantity of seed sown, is one of the simplest means by which the silviculturist may influence the regeneration outcome.

If eucalypt seed is sown at the wrong time time adverse weather may strengthen dormancy, or even kill seed (Grose 1963). Consequently, even when suitable weather and available seedbed later coincide the lack of viable seed will prevent successful regeneration of the site. If seed is sown too late after seedbed preparation, the number of favourable microsites for seed may be restricted, and many of the seeds may fail to germinate. Finally, if seed is sown when conditions are too cold or dry, germination may not occur for months, during which time seed may be lost due to factors such as fungal attack (Cunningham 1960, Mount 1979); insect predation (Cremer 1960; Cunningham 1960; Ashton 1979, Anderson and Ashton 1985); seed wash and burial (Campbell and Bray 1987); or seed germination may be initiated but germinating seeds or seedlings subsequently killed.

While the fraction of seed that germinates determines the initial size of a population, it is principally the phenology of seedling emergence that determines subsequent population dynamics (Silvertown 1982). Ultimately, the success of reafforestation will be determined by the fate of seedling cohorts. The timing of germination is controlled by the physiological processes in the seed that control dormancy and the seed's interaction with environmental factors (such as temperature, moisture, aeration and light: Beardsell and Richards 1987). As a result, different sowing times give rise to different patterns of emergence (e.g. Cunningham 1960; Fagg 1981), and it is clear from work with *Eucalyptus* (e.g. Grose 1957a; Cunningham 1960; Cremer 1962; Fagg 1981; Campbell and Bray 1987) and work with other plant taxa (e.g. Gross 1980; Mack and Pyke 1983; Fowler 1988) that the probability of seedling survival is often associated with the time of emergence. It is also clear (e.g. Daubenmire 1968; Keenan and Candy 1983; Klemow and Raynal 1981; Mack and Pyke 1983; Bowman 1984) that some years favour establishment and others do not, and that consequently, there is a pronounced stochastic element in the outcome of reafforestation efforts.

A generalised representation of the passage of seeds from the time of seed fall through to the next regeneration event, with an indication for eucalypts of the proportion of propagules surviving at each stage (generalised from: Cunningham, 1960; Campbell and Bray, 1987; Lockett, 1991), is given in Fig 1.1. The question of identifying the optimum time to sow seed in native-forest regeneration operations is, therefore, a complex question of plant demography and seed budgeting. The timing of inputs into the ground seed store, the interaction of the ground seed store with biotic and abiotic factors, the phenology of emergence and the survival and growth of seedlings following emergence all influence the outcome.

1.3 Past Research and Current Prescriptions

Despite the complexity of the interactions that influence the success of different times of sowing, prescriptions for native forest regeneration in Australia have been based predominantly on field experiments in which only the net result is recorded and little attention is paid to causative factors.

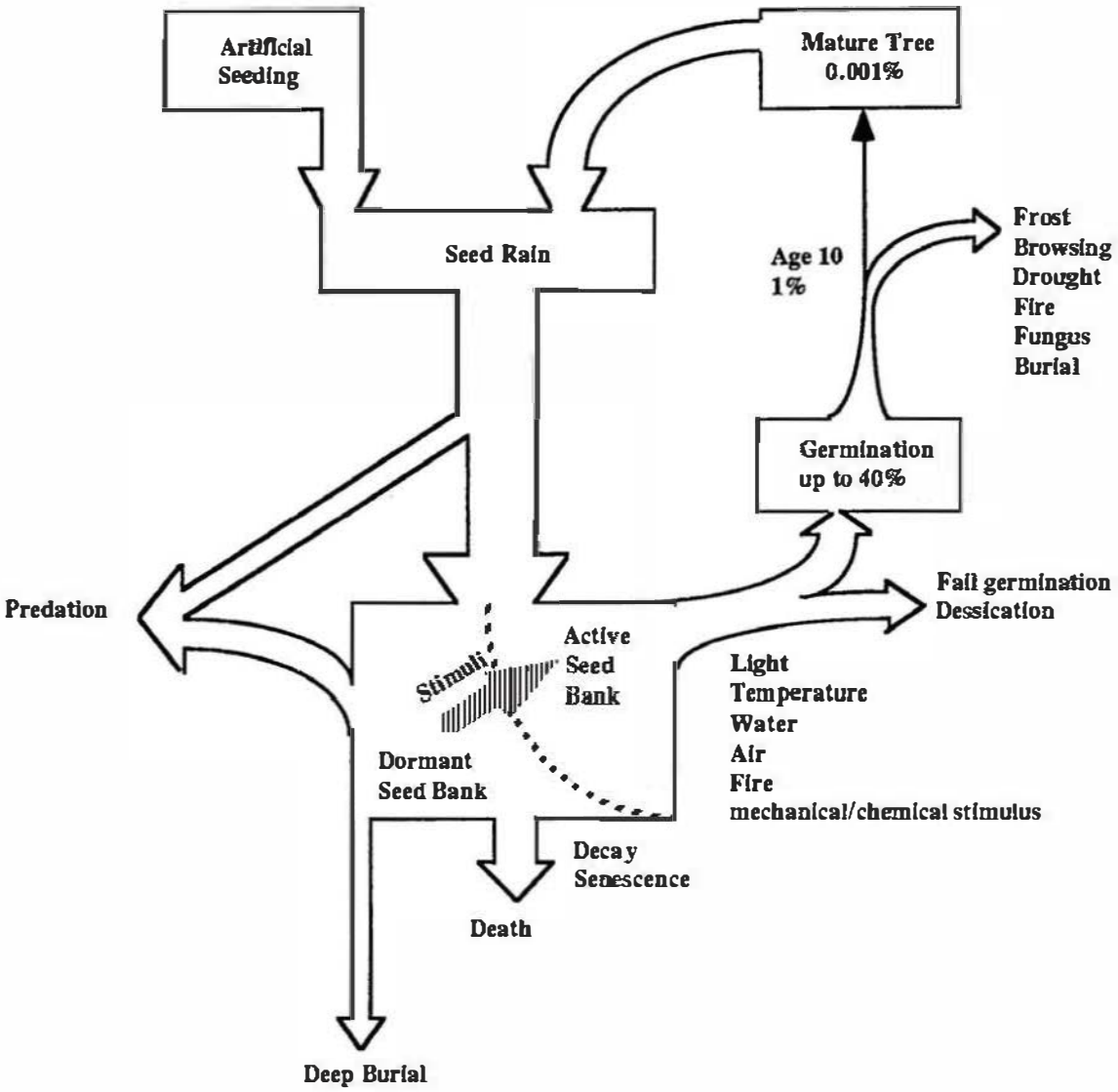


Fig. 1.1. The passage of seeds from the time of seed fall through to the next regeneration event, with an indication for eucalypts of the proportion of propagules surviving at each stage (modified from Silvertown 1982).

Experimental studies into the success of different times of sowing for artificial regeneration, and from studies of natural regeneration events, have made it clear that similar sowing or seedfall times may result in substantially different outcomes in different years. Nevertheless, the limited replication in time of experiments, rather than clarifying the most probable outcome from alternative sowing times, merely highlights the potentially confounding influence of seasonal variation. For example, Grose (1957a) found that in 1954 following a dry spring, most natural regeneration of *E. delegatensis* did not occur until late October and early November. By contrast, spring 1955 was wet, and even though the snow lay on the ground later than in 1954, most seed had germinated by late September. Similarly Fagg (1981), working in the high altitude mixed species forests of East Gippsland, Victoria, found that a dry autumn substantially altered the pattern of emergence in the second year of a series of sowings relative to the first year. In contrast to Fagg's results Johnston (1972) working in the same forest type ten years earlier encountered a drought year and obtained quite different results. Sowings of *E. regnans* in the Central Highlands of Victoria by Cunningham (1960) at the same time in successive years (11/10/1956 and 12/10/1957) gave similar results. However, differences in the timing and pattern of emergence were detected, and unlike the work of Powles (1940) and Campbell and Bray (1987) who found spring the superior sowing time, no difference in the outcome from spring and autumn times of sowing was found. Tasmanian sowing trials replicated over two years in the Florentine Valley (FCT Research Project No. 34 and 34a, cited by Gilbert 1958, 1960 and Cremer 1962) gave a similar ranking of sowing times from the successive sowing years, however the pattern of response within years was highly irregular. A 1955/56 series of sowings resulted in alternating good and bad months for sowing (September and October were good, November was poor, December was successful, January was a failure, February was successful but March was poor, April was good but May was poor). Clearly small variations in conditions within seasons were having a substantial impact on the outcome. Without a better understanding of the interaction of germination physiology and environmental conditions it is difficult to ascertain whether the consistency of response over the two sowing years is coincidental.

The importance of time since seedbed preparation has been commented upon in many time-of-sowing experiments (e.g. Cunningham 1960; Johnston 1972; Campbell and Bray 1987). How the effects of time of sowing and time since seedbed preparation were separated is, however, unclear. In all these works the effect of the time of sowing is confounded by a time-since-seedbed-preparation

effect because, with the exception of Powles (1940), sequential sowings were made onto a seedbed prepared on the one date. It is known that seedbed receptivity declines rapidly and that this can adversely affect seedling establishment (Lockett 1991). The converse is also true with the favourably short interval between seedbed preparation and autumn sowings potentially biasing results.

In addition to limited replication in time of sowing experiments, there has only been limited spatial replication. The entire forestry research effort into the time of sowing of eucalypt seed in south-eastern Australia has been confined to wet forest. Since the advent of the export woodchip industry in Tasmania in the early 1970s, forestry activity has spread into drier areas. Even though the clearfelling of such dry sites is now the exception rather than the norm, clearfelling, particularly of forests intermediate in character between the wet forests of the Florentine Valley and the dry, lowland types of the east coast, is still common. Experience in mine-site rehabilitation and experimentation on agricultural land suggest that on dry sites the factors determining regeneration success are likely to be different to those affecting success in wet forest areas. For example, it was found that the best time for sowing *E. tetragona* on mine tailings in the hot, dry conditions of Eneabba, Western Australia, was May and that spring sowings were unsuccessful because there was generally insufficient time for seedling establishment before the hotter, drier months (Osborne *et al.* 1986; Osborne 1988). On dry pastoral lands in Victoria and South Australia, better weed control in the spring has resulted in spring being the preferred sowing time (Sharp 1985; Weatherly 1985; Oates and Clarke 1987; Geard 1987; Bird *et al.* 1990), although in drier areas (Campbell *et al.* 1988; Dalton 1990) or when a dry spring follows sowing (Pinkard 1992), winter sowing is preferable. Autumn sowing may result in adequate germination but mortality is frequently high (Bird *et al.* 1990). While the weed problems that have influenced practices in pastoral situations will be less pervasive in native forest areas, the difference in factors affecting germination and establishment success raise doubts about the portability of research findings from wetter forest types to drier situations.

Sowing-time prescriptions for native forest regeneration in both Victoria and Tasmania seem to be only loosely related to research findings. In Tasmania, current prescriptions for forestry practice are in most instances for sowings to be made in early autumn (and more recently late winter : Lockett 1991), despite the only local research indicating late winter and spring sowing as suitable. In Victoria there is an increasing emphasis on seed-tree retention for seed supply,

with induction of seedfall following logging (Campbell *et al.* 1990). Where seed-trees are not used, seed is generally sown early in autumn (e.g. Ritchie 1975; Campbell and Bray 1987), even though, for *E. regnans* at least, two out of three of the research programs have found spring to be the superior sowing time. In the high elevation mixed species forests of East Gippsland, these prescriptions have not been successful, and seedfall from seedtrees in the autumn is now supplemented by spot-sowing of residual seedbed in the subsequent spring (S. Murphy, Silvicultural Research Officer Dept. Conservation and Resources, Vic., pers. comm.). Both studies in this forest type (Johnston 1972; Fagg 1981) found this to be a particularly unsuccessful time of sowing. The disparity between research findings and prescription is of concern. The problem is further exacerbated by the failure of operational practice to conform to prescription. This may be partly a result of conflicts between silvicultural objectives and operational constraints. Where seedbeds are produced by high intensity slash burns, particularly on steep country, burning opportunities may be severely restricted. Field managers may be caught between the conflicting objectives of sowing freshly-prepared seedbeds or sowing at the silvicultural optimum time. Because sowing is now done routinely using aircraft, poor weather and aircraft availability further constrain sowing times (Lockett 1991).

Silviculturist and restoration ecologists will continue to require precise quantification of silvicultural options. Unless a more fundamental approach to the question of sowing time is undertaken, every time reafforestation activities are applied to a new area or harvesting regimes, or seedbed preparation techniques are modified, doubts about the validity of current prescriptions will be raised, and substantial and costly field experimentation will be required. Furthermore, a critical component of any management decision is an assessment of the risk inherent in alternative courses of action. Even if the weather during the years which field trials are undertaken is close to average, an assessment of the probability of failure (i.e. the between year variability in outcome) of different sowing times can only be guessed. Rehabilitation costs following regeneration failure are often high (Forestry Commission, Tasmania, 1991) and it may be prudent to sow at a slightly sub-optimal time if the chance of failure is diminished. A quantification of the silvicultural costs incurred in sowing at times outside the identified optimum may result in a better balance between operational expedience and silvicultural objectives.

Finally, because regeneration outcomes are so uncertain, field sowings are heavily loaded with 'insurance' factors (Lockett 1991). Seed, however, is the

most expensive component of the regeneration program, up to 30% of total costs. In many instances the economics of native forest silviculture, particularly in dry forests where volumes per hectare are low and sawlogs comprise a relative low proportion of the timber volume, are at best marginal for the government. Savings made at the commencement of a 60 - 80 year forestry rotation become substantial when compounded over the rotation time, and may influence markedly the economics of native forest management. Similarly, the high cost of seed, and the low ratio of seedlings successfully established to seeds sown, restricts the use of direct seeding (i.e., direct application of seed onto prepared seedbeds) to regenerate trees in pastoral situations in Tasmania and has led to the planting of nursery-raised seedlings in preference (Pinkard 1992). Where establishment of seedlings by direct seeding has proven more successful, the method has become established as a cheap and rapid means of reafforestation. With thousands of hectares of rural land in Tasmania suffering from some form of land degradation, establishment of shelterbelts and woodlots by direct seeding may provide the only feasible means of restoration.

It is probably harsh, but not unfair, to say that the current state of sowing time prescriptions for eucalypts in south-eastern Australia is still probably best summed up by old bush 'recipe', "...half a pound (of seed) for the trees, half a pound for the ants and half a pound for luck" (Youl 1986). New, and more sophisticated, techniques to predict sowing time outcomes are clearly necessary.

1.4 The Use of Modelling to Explore Seed Germination

Despite inherent variability, seeds respond predictably to environmental stimuli. This regularity of behaviour provides the opportunity for modelling and prediction of responses. Because of the comparative simplicity of the germination process relative to many other biological systems, and because successful sowing of crops has been so intimately associated with human welfare, seed germination has attracted considerable modelling attention.

1.4.1 Temperature response

The thermal-sum approach has been used for two and a half centuries to study plant development (see Wang 1960 for review). The technique assumes that a certain physiological stage will be reached after the accumulation of a certain number of day-degrees or heat units. This methodology has been used to model seed germination rates under variable temperature regimes (Hegarty 1971;

Wagenvoort and Bierhuizen 1977; Gracia-Huidobro *et al.* 1982; Dwyer *et al.* 1990). Although such models are useful in describing the germination rate of seeds, they have been less useful in describing germination capacity. By modelling the distribution of thermal times within the seed population, and population distributions for thermal minima and thermal maxima of germination, some workers have managed to describe the time course of germination for some species germinated under isothermal conditions (Washitani and Takenaka 1984; Washitani 1985; Washitani and Saeki 1986). Judicious interpretation of parameter values have in some instances allowed speculation on physiological processes (e.g. Washitani 1985). Murdoch *et al.* (1989) were able to predict variation in germination capacity response diurnal temperatures that fluctuated regulary. However, they were unable to provide information on the time course of germination, and the model's ability to predict germination under non-periodic temperature regimes was not tested. Graves and Taylor (1988) used a thermal-sum model to predict the cumulative germination of two alpine species in the field. Predictions from their model often underestimated field germination, indicating that germination was perhaps enhanced by fluctuating temperatures in a way not considered in the model.

Thermal-sum germination models satisfactorily describe the rate of germination when temperature is the only limiting factor to germination. However, if seed is dormant, this approach will not successfully predict cumulative germination. By increasing the complexity and allowing for factors that relieve dormancy, such as the accumulated chill days (Landsberg 1974; Cannell and Smith 1983; Benech-Arnold *et al.* 1990) or the amplitude of temperature variation (Murdoch *et al.* 1989), the predictive ability of these models can be improved. Such changes, however, typically allow only uni-directional change in seed state, so for example in the model of Benech-Arnold *et al.* (1990), dormancy could only be relieved and not induced. Further, the approach assumes that the rate-limiting physiological process responds in a linear manner to temperature. This is at best an approximation over a limited range of temperatures, since most biological temperature responses are sigmoidal in form at low to moderate temperatures followed by a rapid drop in rate above a temperature optimum (Johnson and Thornley 1985). The linear model possibly fits in certain cases because the combined action of temperature sensitive and temperature insensitive processes, within the domain of experimental conditions, changes the overall form of the response (Washitani and Takenaka 1984).

Physiologically-based, or semi-mechanistic, models of germination response to temperature have been rarely attempted. The limited knowledge about seed physiology, and the probable complexity of the germination process, virtually negates the development of a truly mechanistic model at present (Thornley 1986). Hageseth (1974) and Hasgeth and Joyner (1975) developed a model based on the autocatalytic reaction model that describes biochemical reactions in which enzymes play an important role. This model, which successfully described the germination rate and the germination capacity of test seedlots, was only ever tested under constant temperature conditions, although there is no apparent reason why it would not have been successful under variable conditions. Thornley (1986) developed a semi-empirical model to describe the time course of germination. While no data were fitted to the model, he was able to demonstrate that it could assume a wide range of forms. This model, because of limitations inherent in its structure as a linear compartmental model, did not allow for changes in the dormancy characters of the seed population during the germination period since seeds adversely affected by high or low temperatures in this model were considered to be killed.

1.4.2 Water stress response

The overall response of germination to water stress has received less attention by modellers, although the response of particular aspects of the germination process has been studied in detail. The imbibition process in particular has received considerable study (e.g. Dewez 1964; Phillips 1968; Blacklow 1972; Wanjura and Buxton 1972). In some instances, by relating time to emergence to time to complete imbibition, good predictions of field emergence have been achieved (Hadas 1977*a*). The effect of water potential on hypocotyl and radicle extension has been thoroughly investigated (e.g. Cleland 1967; Hegarty and Ross 1978) and has been successfully modelled (Wanjura and Buxton 1972). Despite this abundance of physiological information, few attempts have been made to model field germination response under conditions of limiting soil moisture. Generally, studies have been made either when soil water is non-limiting or seeds have been irrigated (e.g. Hegarty 1971; Wagenvoort and Bierhuizen 1977). McKeon *et al.* (1985), however, by modelling the rate of dehydration of seeds were able to predict the required rainfall event necessary to trigger successful germination of a desert annual in a monsoonal climate. Despite the known interaction of temperature and water potential on seed germination (e.g. Lindstrom *et al.* 1976; de Jong and Best 1979; Livingston and de Jong 1990) few studies have tried to model the effects of temperature and water potential simultaneously. Indeed few

experiments examining plant physiological performance have simultaneouly manipulated water potential and temperature (Feng *et al.* 1990). Exceptions are Washitani and Saeki (1986) who investigated the effect of dehydration during the imbibition process on the total thermal time required for germination and Lindstrom *et al.* (1976) who used an empirical relationship to predict the combined effect of temperature and water potential on the emergence time of winter wheat. Lindstrom *et al.* (1976), however, concluded that because processes of germination (imbibition, radicle emergence, and subsurface seedling elongation) differ considerably in sensitivity to soil environmental factors, a more fundamental approach was preferable to their empirical function.

1.4.3 The role of modelling

The successful mechanistic modelling of eucalypt germination in the field will provide a means of avoiding many of the pitfalls associated with identifying the optimum time of sowing of eucalypt seed in reafforestation activities. The modelling process will provide a means of testing hypotheses concerning the causal relationship between seasonally-influenced weather patterns and cumulative germination. In addition modelling will provide the silviculturist or manager with a quick means of anticipating the implications of combinations of sowing and seedbed preparation timings. The manager will be able to evaluate the potential outcomes and strike an appropriate balance between expedience, practicality and the achievable yield.

However, serious doubts have been expressed about whether modelling exercises are particularly cost-effective, efficient or useful means, as their proponents claim, of synthesising knowledge, testing hypotheses, creating conceptual frameworks to guide future research or of generating new hypotheses (Passioura 1973; Simberloff 1981; Ulanowicz 1988). These arguments are not without substance. The literature of seed germination modelling abounds with "black boxes" and assumptions which are ultimately untestable. For example, the family of models based on the work of Gracia-Huidobro *et al.* (1982) all rely on the presumption, untested, and virtually untestable, that the same sub-population of a seed sample will always germinate first under a given set of conditions.

If such criticisms are to be avoided, assumptions embedded in the mathematical formulation should be testable and stated explicitly. As far as possible the internal functioning, as well as the gross output from the model, should be open to validation. The model should be kept comparatively simple and the

parameters few enough to be measured and tested directly or indirectly. Some problems and some systems, however, are more appropriately explored using mechanistic models to predict outcomes than are others. Systems, like seed germination, in which a single population or process that displays little biological autonomy and is strongly dominated by physical influences, are clearly the most suitable (Ulanowicz 1988). In addition, the driving variables that influence seed germination are easily measured, and the system's state over time is easily quantified. There is a substantial body of literature dealing with mechanisms of germination and germination responses which can be drawn upon in the systems analysis of the germination process. If this proves inadequate, seed germination response to combinations of environmental factors is comparatively easily measured in experimental systems. Nevertheless, many iterations of system characterisation, mathematical formulation, validation and additional data collection will be necessary before the fundamental aspects of seed germination in the field are mimicked and a model of sufficient robustness and generality for widespread application is developed. Although this is an involved process, it may, in the long run, prove more fruitful and focussed than the repetitive and fragmented research on eucalypt time of sowing that has been carried out to date.

1.5 Ecology and seed germination response of *Eucalyptus delegatenis*

This thesis undertakes the development of a mechanistic germination model to predict the emergence of *E. delegatensis* under field conditions. *Eucalyptus delegatensis* germination response to environmental stimuli is more complex than most other commercially exploited eucalypts in south-eastern Australia (Boland *et al.* 1980). A germination model that successfully predicted the emergence of *E. delegatensis* in the field would, with minor modification, be sufficiently complex to explain the germination response of a wide range of species for which the principal determinants of field germination were temperature and soil moisture.

1.5.1 Natural distribution of E. delegatensis

Eucalyptus delegatensis occurs in the south-eastern states of Australia where it is known by a number of common names: alpine ash (New South Wales and Victoria), woollybutt (Victoria) and gum-topped stringybark (Tasmania). The Tasmanian provenances of *E. delegatensis* have long been noted as different from mainland provenances, and have, in the past, been described as a separate species, *E. gigantea* Hook.f. (Hooker 1847, 1856). Subsequent work has shown

that the morphological variation in many characters between populations on the mainland and in Tasmania is continuous, but marked differences in two characters, the presence or absence of verrucose stems and the morphology of the seedling and juvenile leaves (Boland and Dunn 1985) has led to the recommendation for the recognition of two geographically separated subspecies (Boland 1985). Other differences have also been noted between mainland and Tasmanian provenances, most particularly the greater fire resistance and the greater propensity for lignotuber formation amongst Tasmanian provenances (Bowman 1984).

In Tasmania, the species occurs over a broad environmental range: it is found over an altitudinal range of between 300 - 1300 metres above sea-level; occurs in areas with rainfall rainfall ranging from 700 mm/year to areas where the rainfall exceeds 2500 mm/year; can be found on a variety of substrates derived from lithologies including dolerite, basalt, granite, sandstone, quartzite, schists and porphyry (Boland *et al.* 1984; Ellis and Lockett 1987); and occurs in every Nature Conservation Region (described in Orchard 1988) on the mainland of Tasmania (Williams 1989). A generalised map of its distribution in Australia and a detail of its occurrence in Tasmania is given in Fig. 1.2.

1.5.2. Phytosociology of E. delegatensis in Tasmania

Eucalyptus delegatensis generally occurs in pure stands or as a dominant species in association with other species. It is associated on frosty or very cold sites with *E. pauciflora, E. dalrympleana, E. gunnii* and *E. coccifera,* and in the south-east with *E. urnigera.* Towards the drier end of its range it is often associated with *E. amygdalina* and in the south-east of Tasmania occasionally with *E. pulchella* and *E. tenuiramis.* At the wetter end of its range it may be associated with *E. subcrenulata.* Towards the lower altitude limits of its range it becomes associated with *E. obliqua* and *E. globulus* in ecotonal forests. The principal environmental parameters related to forest structure are fire frequency and rainfall (Bowman 1984; Ellis 1985; Duncan and Brown 1985; Kirkpatrick *et al.* 1988). Where the annual rainfall exceeds 1250 mm and the fire frequency is low *E. delegatensis* is typically emergent over rainforest. Frequent disturbance, such as partial logging, or frequent fire may result in an understorey of rainforest shrubs such as *Tasmannia lanceolata, Persoonia gunnii* and *Comprosma nitida,* and a ground cover of *Poa* spp. (*P. gunnii, P. rodwayi* and *P. labillardieri*) and ferns. Very frequent fires, particularly in association with areas of cold air accumulation such as plateau sites, can lead to the removal of virtually all

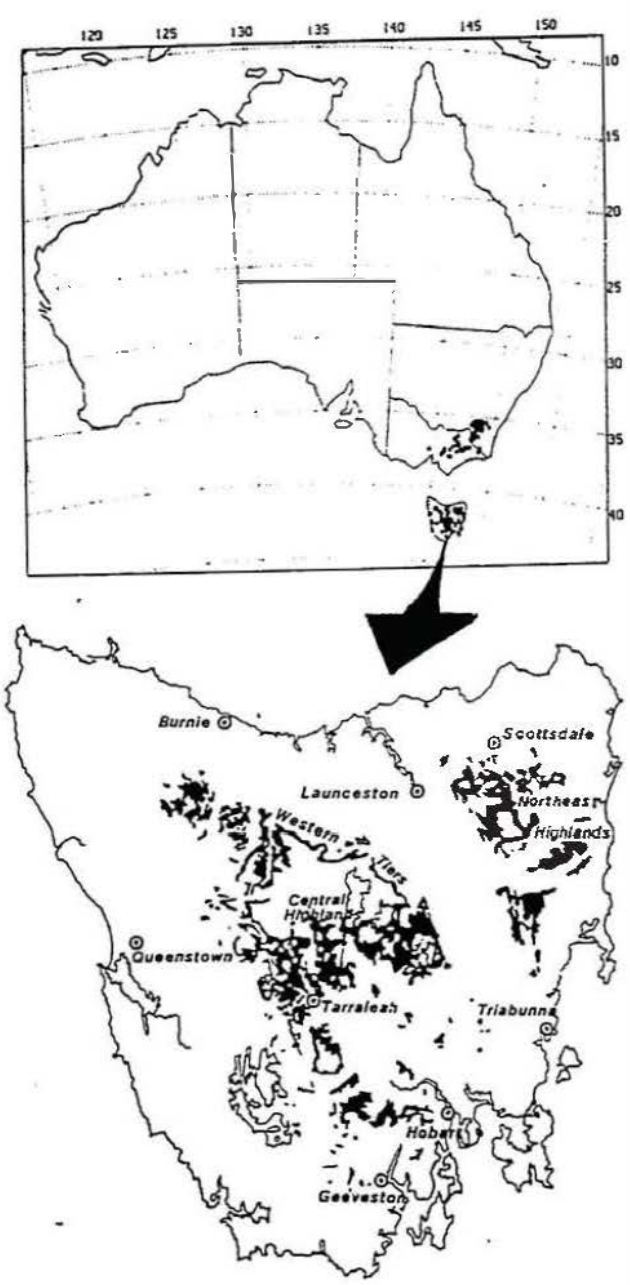


Fig. 1.2. The natural distribution of *E. delegatensis*. The map of Australia is reproduced from Boland *et al.* 1984 and the map of Tasmania from Battaglia 1991.

understorey species and a ground cover consisting almost exclusively of *Poa* spp. At lower altitudes where the annual rainfall is below 1250 mm, *E. delegatensis* may occur with a wet sclerophyll understorey consisting of such species as *Bedfordia salicina, Zierra arborescens, Olearia argophylla* and *Pomaderris apetala.* At higher altitudes where the annual rainfall is less than 1000 mm a heathy understorey dominated by *Cyathodes parvifolia, Leucopogon hookeri, Lissanthe montana, Lomatia tinctora* and *Pultanaea juniperina* may be present. These forests frequently have tussock-forming grasses and sagg species present in the understorey, and in some instances these can form the principal ground cover. As a lowland dry sclerophyll species it occurs mainly over shrubby understories. These include many species in common with higher altitude dry *E. delegatensis* forest, but also include species such as *B. salicinia, Acacia dealbata* and *Pimelea nivea.*

1.5.3 Regeneration processes

1.5.3.1 Natural regeneration

Eucalyptus delegatensis, in common with most eucalypts, requires a disturbed seedbed for successful regeneration (Grose 1957a; Bowman 1984). Although windthrow (Gilbert 1959) and landslides (Mount 1979) may create small areas of disturbance in which germination may occur, fire is overwhelmingly the most important agent of disturbance. Natural regeneration events are, therefore, almost inevitably associated with wildfire events.

On only two or three days per year in south-eastern Australia are conditions in mixed or wet forests conducive to the extensive spread of fire (Ashton 1981). Generally, the high, reliable and even seasonal distribution of rainfall and the protection of ground fuels from drying by the dense understorey canopy reduces the fire hazard. It is only when there is a coincidence of ignition source, severe fire weather and prolonged drought that widespread fires can develop. Fires are, therefore, largely restricted to late summer and early autumn, only occasionally occurring in early summer and mid-autumn. Because these fires are fuelled by the heavy understorey extensive canopy damage usually results. Research in an ecologically and phylogenetically related species, *E. regnans,* indicates that capsules dry out quickly after such canopy damage and shed seed in a single event commencing within two days of the wildfire and complete within a month (Cunningham 1960; Cremer 1962). Up to half a million seeds per hectare can be shed in this period (Grose 1957a; Cunningham 1960; Ashton 1976, 1979;

Harrison *et al.* 1990). For wet forests the natural "time of sowing" is, therefore, predominantly late summer and early to mid-autumn.

In the drier forests fires are more frequent and usually less intense. Death of mature trees, and replacement by regeneration is usually the net result of successive wildfires. Locally intense fires associated with fuel accumulated around the boles of trees may give rise to fire scars where cambial tissue has been locally killed. Repeated burning may deepen these wounds and ultimately destabilise the tree. Additionally insect attack, especially the termite, *Porotermes* (Elliott and Bashford 1984), stemming from fire wounds, may kill or fell the tree. This in turn may give rise to a local accumulation of fuel. A subsequent fire will be locally intense with a long residence time and will generate an ashbed (Mount 1979; Vines 1968; Jacobs 1955) suitable for seedling establishment and growth (Pryor 1960; Attiwill 1962; Renbuss 1968; McCormick 1990). The combined stimuli of the ashbed and the canopy gap arising from the felled tree may lead to the recruitment into the canopy of germinants arising after the fire, or the release of nearby suppressed lignotuberous seedlings (Henry and Florence 1966; Bowman 1984; Incoll 1979; Kellas *et al.* 1982, 1987). Unlike wet forests, conditions in many areas of dry forest may be suitable for the spread of wildfire from the beginning of October to the end of April. Low intensity fires do not always induce seed shed. Regeneration opportunities in such cases are delayed until natural seed shed occcurs in late summer and autumn (Grose 1957a). However, if fires are intense, seed fall may occur immediately following the fire. Hence, in dry forest the natural "time of sowing" can extend through 6 months of the year, although, similar to wet forest, it is probably predominantly concentrated in the autumn.

1.5.3.2. Artificial regeneration

In all three south-eastern states *E. delegatensis* is an important commercial timber species. In Tasmania, it constitutes a major proportion (12%) of the state's forest resource (Ellis and Lockett 1987). *Eucalyptus delegatensis* forests have a long history of forest management in Tasmania and have proven remarkably amenable to a range of silvicultural treatments, including clearfelling, partial logging and single-tree selection (Ellis and Lockett 1987; Battaglia 1990). Currently 80 per cent of the area of *E. delegatensis* forest logged in Tasmania is regenerated using some form of partial logging, predominantly uneven-aged management systems, with a small area managed under shelterwood regimes (Graham Wilkinson, Principal Research Officer Native Forests, FCT, pers. comm). The areas of forest

clearfelled are mainly wet sclerophyll and mixed-forest stands. Where they exist, established lignotuberous seedlings are allowed to grow on following logging. In partial-logging systems, seed fall from retained trees is mostly relied upon for additional regeneration. Clearfelled areas, large gaps created in partially logged coupes, and areas where seedtrees lack viable seed crops, are artificially seeded. If coupes are left unburnt, fallen trees may shed seed within weeks of felling, and as a consequence seed fall may be distributed over almost the entire period of the logging operation. However, if seed trees are retained, seedfall will occur predominantly in the last weeks of summer and the first weeks of autumn. In artificial sowing operations approximately 70 000 viable seeds are sown per hectare, though if sites are classed as difficult to regenerate this is increased (Lockett 1991). The Forestry Commission, Tasmania aims to complete sowing by the end of March (Lockett 1991). In practice sowing often continues into May, and may be delayed until the following spring.

1.5.4 Requirements for seed germination

The requirements for seed germination of the majority of species in the Myrtaceae are easily satisfied: provided seeds are given adequate moisture, warm temperatures, oxygen and, in many instances, light, germination is rapid (Turnbull and Doran 1987). *E. delegatensis*, along with a number of other high altitude members of *Eucalyptus*, is an exception, often requiring cool, moist conditions (stratification) for complete germination of a seed sample.

1.5.4.1 Gases

Grose (1963) found that excising the embryo or rupturing the inner and outer seed integuments promoted rapid and full germination of *E. delegatenis* seeds. Increasing the partial pressure of oxygen increased germination, and decreasing the partial pressure of oxygen, by increasing the carbon dioxide supply, decreased germination. Poor aeration, achieved by immersing seeds in water, reduced germination. Dexter (1960) found that increasing the availability of oxygen to the embryo by applying hydrogen peroxide broke the dormancy of some seeds.

The changes in atmospheric gas partial pressures required to bring about significant changes in germination are large and apart from the obvious implications of prolonged water-logged soil, it is unlikely that the partial pressure of oxygen will influence field germination significantly.

1.5.4.2 Water Potential

There is no work specifically investigating the germination response of *E. delegatensis* to soil water potential. Seed germination of other members of the *Eucalyptus* subgenera *Monocalyptus* are sensitive to small changes in soil water availability (Zohar *et al.* 1975; Edgar 1977; Bachelard 1985; Gibson and Bachelard 1986*a*). These experiments, however, indicate that the response of seed is highly sensitive to the way water stress is applied. When moisture stress is applied by exposing seeds to solutions of differing osmotic potentials both the per cent of seed that germinates and germination rate are affected only slightly by osmotic stresses above -0.1 to -0.2 MPa (Edgar 1977; Zohar *et al.* 1975). When stress has been applied using tensionmeters, for example by the Haines' Apparatus (Gibson and Bachelard 1986*a*), far less water stress, in the order of -0.003 MPa, impedes germination. Comparison of methodologies on the samples of the same seedlots by Bachelard (1985) confirmed this difference.

Different eucalypt species have been found to respond to soil moisture stress in different ways (Edgar 1977; Bachelard 1985; Gibson and Bachelard 1987). The differences are related in part to seed size and seed-surface characteristics (Bachelard 1985; Gibson and Bachelard 1987). Such differences in seed physiology, and hence ability to germinate under stress, have been related to the geographical distribution of species (Edgar 1977, Bachelard 1985) and variation between provenances within species have been related to site and climatic factors where provenances naturally occur (Gibson and Bachelard 1987).

Seed of *E. sieberi*, a species that occurs in more drought prone environments than *E. delegatensis*, is able to withstand intermittent drying during germination, and still germinates after five wetting and drying cycles (Gibson and Bachelard 1988). Gibson and Bachelard (1986*b*) found that seeds must maintain a water content equal to 30 per cent of dry weight for at least 60 hours to commence germination. An ability to achieve this water content depends on the atmospheric humidity (Gibson and Bachelard 1986*a*, 1986*b*) and the degree of contact between the seed and the substrate (Gibson and Bachelard 1986*b*) as well as soil matric water potential.

1.5.4.3 Light

Light quality may significantly influence the germination capacity of *E. delegateniss* seeds. MacLeod (1981) found that the germination capacity of test

seed was greater in the dark than under an incandescent light source, a fluorescent light source or a mixture of the two. This work found that germination under red light was equal to germination in the dark. Germination under far red light was inhibited, suggesting the action of a phytochrome system. In common with other eucalypts (Grose 1965), it has been found that stratification reduces the sensitivity of germination to light quality (MacLeod 1981).

Light quality, particularly the red/far red ratio, varies over the course of a day and seasonally with the sun angle (Monteith 1973). The annual variation in light quantity and quality at the soil surface, within a clearfelled coupe, is probably small relative to the sensitivity of *E. delegatensis* seed. The preferential germination of seeds in the dark may, however, be an indication of a positive response to partial or complete seed burial. Nevertheless, light is unlikely to be a critical factor in determining the optimum time to sow seed, although it may indicate the importance of sowing promptly after seedbed preparation.

1.5.4.4 Dormancy and Temperature Response

Stratification of *E. delegatensis* seed promotes germination of the dormant proportion of a seedlot and increases the germination rate of seeds (Pryor 1954; Grose 1957*a*, 1963; MacLeod 1981). Considerable inter-provenance variation exists in the proportion of dormant seed (Grose 1963; Boland and Dunn 1985). From 246 germination tests E. J. Lockett (unpub. data) has found that Tasmanian seed is 46 $\pm$ 23 per cent dormant. By contrast, Grose (1963) found from 87 tests that Victorian seedlots are 79 $\pm$ 14 per cent dormant. Grose (1963) found no apparent variation in the degree of dormancy with elevation among Victorian provenances (a result later confirmed by Abbott and Pederick (1984)) or aspect, and no difference in seedlots from widely separated localities. Trees growing in small compact groups were, however, shown to differ significantly in the degree of seed dormancy. Boland and Dunn (1985), however, postulated the presence of a number of geographical sub-populations of markedly different dormancy characteristics. The two mainland sub-populations were identified as having 89 and 69 per cent of seeds dormant, compared with 15, 34 and 36 per cent dormancy in the Tasmanian sub-populations.

Dormancy is removed by stratification in the temperature range 1°C - 10°C. Dormancy release is faster at higher temperatures, but above a threshold, possibly around 8°C, longer periods of storage begin to reimpose dormancy. Stratification

for one to two weeks at 10°C (Grose 1963) and 11°C (MacLeod 1981) removes the dormancy from a portion of the seedlot, although the effect is lessened or reversed after four weeks or longer of stratification. Storage of seed at temperatures or moisture contents unfavourable for germination is capable of strengthening primary dormancy or inducing secondary dormancy (Grose 1963). Seed with a moisture content of below 15% is impervious to exposure to high temperature (32°C) and the rate at which the proportion of dormancy increases when seed is below 21per cent moisture content is slow. Fully imbibed seed, however, suffers strengthening of dormancy at 27°C after as little as eight hours. Prior stratification reduces the strengthening of primary dormancy and slows the onset of secondary dormancy. The occurrence of strengthened primary or secondary dormancy has not been recorded amongst the less dormancy-prone Tasmanian sub-populations under realistic regimes of temperature. MacLeod (1981) demonstrated a reduction in germination capacity of some Tasmanian seed but only after four weeks storage at 32°C.

Most studies have shown that without stratification *E. delegatensis* seed has a temperature optimum for germination in the range 15°C-21°C (Grose 1963; Scott 1972; Boland *et al.* 1980; Davidson and Reid 1980; MacLeod 1981). Stratification increases the range of temperatures over which a high germination capacity occurs so that, after eight weeks stratification at 5°C, seed will germinate almost equally well at any temperature between 5 and 27°C (Grose 1963; MacLeod 1981). Constant temperatures of between 5 and 7°C induces complete germination of the viable fraction of the seed, but at 2°C no germination occurs, even though full germination occurs when the seed is subsequently shifted to a higher temperature (Grose 1963). Alternating temperatures do not significantly improve germination for the species unless minimum temperatures are within the range of stratifying temperatures (Grose 1963; MacLeod 1981).

Although seed dormancy appears sensitive to relatively minor changes in temperature, the seed itself survives over a wide range of temperatures. Cremer and Mucha (1985) showed that air-dried seed is capable of surviving temperatures as low as -32°C without any adverse effects, imbibed seed is unaffected by short exposures to temperatures as low as -6°C, and that half of imbibed viable seed still germinated after exposure to -16°C. Seed also appears quite resilient to high temperature exposure. MacLeod (1981) found that after four weeks storage of imbibed seed at 32°C, 80 per cent still germinated after

stratification, and after four weeks at 37°C, 15 per cent still germinated after stratification. Prolonged exposure to 42°C killed all seeds.

The mechanism of dormancy in *E. delegatensis* is poorly understood. Grose (1963) proposed that restriction of oxygen passage through the inner integument of *E. delegatensis* seed results in competition between respiratory and oxidative processes. He suggested that each process had a different temperature response and that anaerobic respiration occurred when the partial pressure of oxygen within the integument falls below a critical level. He postulated that at low temperatures, (between 1° and 7°C), the rate of both respiratory and oxidative processes are slow and enough oxygen is present to prevent anaerobic respiration. As the temperature rises, the rate of the respiratory processes increases and competition for oxygen becomes critical. It is suggested that at higher temperatures considerable anaerobic respiration occurs resulting in a breakdown of oxidised food reserves back to their initial form, or at least to a form capable of being oxidised again later. This re-oxidisation, Grose (1963) suggested, may occur during further stratification, or as a result of an increase in oxygen partial pressure when the inner integument is ruptured by the hypocotyl during germination.

Grose (1963) attempted to explain the observed germination behaviour of his test seedlots using the following model. He proposed that the rate of reactions in seed that lead to a reduction in dormancy increase from 0° to 5°C, and that this rate is maintained or decreases only slightly between 5° and 10°C, but probably falls rapidly above 10°C. The rate at which dormancy is strengthened increases with temperature and is probably in equilibrium with the first reaction at 10°C. Between 10 and 17°C the rate of the germination reactions increases faster than that which strengthens dormancy so that progressively more seeds can germinate in a fixed time. Above 17°C this relative position of reactions is reversed so that by 24°C the two rates are similar and few or no seeds germinate. Above 24°C the dormancy is strengthened, presumably by anaerobic metabolism of existing food reserves.

In his work on the mechanism of seed-coat imposed dormancy, Grose (1963) draws on work dealing with the nature of dormancy in *Xanthium,* investigated earlier this century (e.g. Crocker 1906, Davis 1930; Crocker 1948, Crocker and Barton 1953). The response of germination to increases in oxygen partial pressure, the increase in dormancy caused by poor aeration, the increase in germination capacity following excision of the embryo and the induction of

secondary dormancy by lowering the partial pressure of oxygen are similar in both species. The contention that dormancy in *Xanthium* is related to poor seed coat oxygen permeability has subsequently been challenged (Black and Wareing 1959, Bewley and Black 1982). These authors contend that water soluble inhibitors are responsible for dormancy in *Xanthium*, and that elevated oxygen concentrations relieve dormancy through enzymic oxidative reactions that lead to a reduction in the levels of these inhibitor substances. The excision of embryos encourages germination, in this model, by permitting the escape of the inhibitor substance(s) and not, in fact, by allowing easier access to oxygen (Wareing and Foda 1957). The possibility of this mechanism working in *E. delegatensis* is supported by the observation that germination of many *Eucalyptus* species is impeded by the leachates from the seeds if they are germinating on a medium (e.g. filter paper) that does not allow movement of these leachates away from the seed (Boland *et al.* 1982). The removal of dormancy by chilling would require that cold stratification assist the removal of these growth inhibitors or increase the level of growth promoters. Bewley and Black (1982) after reviewing the literature regarding this found that although abscisic acid levels decrease with chilling in some species, it is not chilling *per se* that is responsible for the decrease in abscisic acid, and even though a fall in abscisic acid levels may be necessary for the termination of dormancy other changes must also take place. They conclude that abscisic acid is not needed for the maintenance of dormancy and that 'There is little to convince us ... that changes in the inhibitors of seeds are of much significance in the termination of dormancy by low temperatures' (Bewley and Black 1982, pg. 225).

The addition of gibberellin, a growth promoting hormone, has been shown to remove or reduce dormancy effects in eucalypts (Bachelard 1967; MacLeod 1981). Levels of gibberellin have not been measured over the course of dormancy release during stratification of *E. delegatensis* seed. Elevated levels of gibberellin have been recorded for a number of other species during the chilling of seeds (see Bewley and Black 1982) but none has shown that increasing gibberellin levels are responsible for breaking dormancy. In many of these studies the relationship between changing gibberellin levels and germination response does not appear to be well synchronised (e.g. Webb *et al.* 1973; Sinska and Lewak 1977; Pinfield and Davies 1978). It has also been suggested that essential changes may occur in only a limited area of the seed and involve only a small portion of the total hormone level, a change almost impossible to detect (Trewavas 1986).

The concept of a hormonal balance has been more recently rejected as the mechanism controlling germination in seeds with seed-coat imposed dormancy. It has been shown in work with *Arabidopsis thaliana* that it is the inhibitor action (abscisic acid) that determines the onset of dormancy (Karssen *et al.* 1983). The maintenance of dormancy, however, does not appear to be related to the level of abscisic acid. Growth promoters are required for germination, and it appears that the release from dormancy occurs as a result of an increase in sensitivity of seed to the growth promoter, gibberellin (Karssen *et al.* 1989). Gibberellin dose-response studies have shown that gibberellin requirement seems to be proportional to the abscisic acid-induced blockage of germination (Karssen and Lacka 1986). Based on these results it has been proposed that dormancy is controlled "through the simultaneous presence of differing levels of antagonistic hormones by a kind of 'remote control' in which the GA requirement of germination is controlled by the ABA levels during seed development via the intermediate of ABA-induced dormancy ... This intermediate might be the suppression of cell wall extensibility in the elongation zone of the embryos." (Karssen *et al.* 1989: pg. 79).

1.5.5 Seedling survival

By contrast to the intensive experimental study of *E. delegatensis* seed germination response to environmental conditions, the relationship between the timing of seedling emergence and the ability to survive environmentally-induced stress has been examined in much less detail.

In field studies in a range of eucalypt species, mortality resulting from drought has been found, in most years, to be confined to seedlings of the cotyledon or early two-leaf stages, and hence is normally only a major mortality factor for seedlings that germinate in late spring or summer (Cunningham 1960; Cremer 1962). Drought-related deaths of *E. regnans* seedlings germinating in clearfelled coupes in autumn for example, have been reported at below 10% (Cremer 1962), however, following spring sowings in the high altitude forests of East Gippsland, Victoria, far higher mortality has been reported (Fagg 1981). Only severe droughts have been observed to kill large trees (e.g. Kirkpatrick and Marks 1985; Davidson and Reid 1989).

Frost heave only occurs with the coincidence of saturated soils and severe frosts and is most common in winter (Cremer 1962; Campbell and Bray 1987). Normally only those seedlings that germinate in late autumn and in winter are

small enough to be affected (Cremer 1962, Fagg 1981; Campbell and Bray 1987). The effect can, however, be severe and in some studies up to 25 per cent of seedlings originating from an autumn sowing have been reported to have died after frost heave during winter (Cremer 1962; Campbell and Bray 1987). In an average year, frost heave is probably a far more significant mortality factor among very young seedlings on clearfelled sites than foliar damage as a result of frosts. As seedlings age and develop a more extensive root system, thus increasing their resistance to frost heave, this relationship could be expected to change. Following severe frosts , however, significant numbers of deaths can occur as a result of foliar and stem damage (e.g. Cremer 1962; McKimm and Flinn 1979; Griffin *et al.* 1982). In subalpine valleys with a thick grass sward, in particular, seedling death due to frost is more common (Harwood 1983; Paton 1983; Ashton and Hargreaves 1983; Bowman and Kirkpatrick 1986). Being closer to the ground surface small plants are subject to lower temperatures (Meskimen 1983; Davidson and Reid 1985). Older seedlings and saplings are still vulnerable to very severe frosts (e.g. Calder 1850; Bond 1945; Davidson and Reid 1985). Despite frosts being most severe in winter, seasonal patterns in eucalypt frost hardiness (Hallam and Reid 1989) may mean that comparatively mild frosts on unhardened leaf tissue in the warmer months are just as damaging. For example, Cremer (1962) found that the most severe frost damage in a regeneration trial occurred in February and March, and that relatively mild frosts in late winter and early spring caused substantial mortality when preceded by a short spell of unseasonably warm conditions.

Other mortality factors are usually less significant in artificial eucalypt establishment, but occasionally have been of local importance. Fungal death occurs predominantly when seedlings are at the cotyledon stage, larger seedlings succumbing only when over-topped by weeds (Cunningham 1960; Gilbert 1958; Cremer 1962; Ashton and Turner 1979; Campbell and Bray 1987). In the laboratory the use of fungicide has been shown to result in an eight-fold reduction in germinant mortality over the first few weeks following emergence (Neumann and Kassaby 1986). Defoliation by insects has been found to be most severe in summer and autumn (Cremer 1960; Leon 1989) and when combined with spring sowings has resulted in very high seedling mortality (Fagg 1981). Defoliation is most likely to be fatal to small seedlings, and is generally believed to be most severe in summer and autumn when up to one fifth of all mortality has been ascribed to decapitation (Cremer 1962). Vertebrate browsing has been found to be most severe during autumn and winter when alternative foods are limited (Statham 1983) and may be a locally severe cause of mortality (Gilbert

1958; Cremer 1962). Finally, root exposure following heavy rain has been found to be a significant cause of death amongst very small seedlings (Grose 1957a).

1.6 Structure of this thesis

A typical modelling stratagem is described in Fig. 1.3. This provides a framework within which to view the contents of this thesis. The nature of the problem has been introduced and the prior knowledge about the system reviewed in this chapter. Experimentation to supplement this knowledge and to generate data with which to test subsequent model predictions is described in Chapters 2-6. The germination response of *E. delegatensis* seed to some key determinants of germination is explored. This is the basic information that will determine the form of the subsequent germination model. Variation within and between provenances in these characteristics is investigated briefly. This information will provide information on the specificity of a germination model developed, as well as providing information valuable in developing seed collection strategies. The importance of spatial heterogeneity in seedbed conditions in determining germination behaviour is examined. Such spatial variability will clearly be of importance in predicting field emergence. Changes in the frost sensitivity and drought tolerance of seedlings as they age are investigated to provide insight into the fate of seedlings immediately post emergence. A field experiment designed to investigate the question of the optimum time of sowing is outlined. This is an experiment designed very much along the lines of the typical time of sowing experiments reviewed above. This experiment provides field data with which to validate subsequent modelling of seed germination. A compartmental model of germination is developed and the individual processes described. Parameter values are estimated using the data from the experimental work in the early chapters. The model is validated using test data sets from glasshouse and controlled environmental studies. A predictive model of field soil conditions is developed and this is coupled with the germination model, and monthly estimates of the rate of seed attrition from the soil, to predict cumulative field emergence.

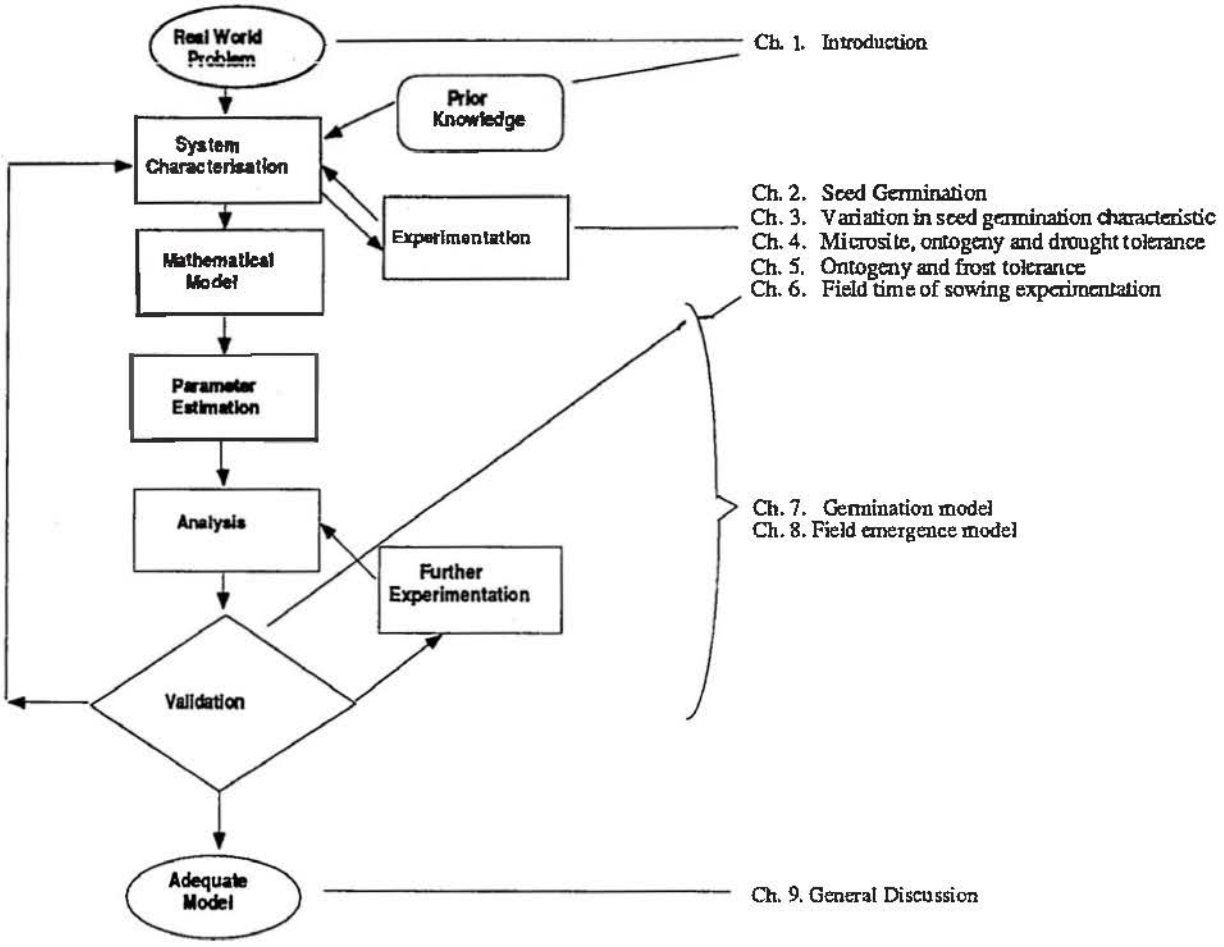


Fig. 1.3. Thesis outline within the context of the modelling stratagem.

Chapter 2

Factors controlling seed germination of *Eucalyptus delegatensis*

Chapter 2: Factors controlling the seed germination of *Eucalyptus delegatensis* R.T.Baker.

2.1 Introduction

Past research into the germination of eucalypt seeds predominantly has described the germination response of a particular eucalypt species to only one or two types of environmental stimuli (e.g. temperature and stratification, Grose 1963; soil water potential and relative humidity, Gibson and Bachelard 1986a). Where a more holistic approach has been taken (e.g. Zohar *et al.* 1975) environmental stimuli have been shown to interact in their effect on germination. A full understanding of the germination of a given species in the field, therefore, requires the investigation of all major environmental determinants of germination and their interaction. This, coupled with an evaluation of intra-species variation for germination responses, would provide the basis for the development of a robust germination model for the species.

The literature review (Chapter 1) has indicated the principal determinants of *Eucalyptus delegatensis* germination in the field are likely to be temperature and soil moisture. Although the temperature responses of *E. delegatensis* have been examined for mainland provenances, the known difference in dormancy attributes of Tasmanian provenances suggests it would be prudent to examine Tasmanian provenance response. The response of the species to water stress is virtually unknown. Work with *E. sieberi*, however, suggests that as a minimum the responses to matric stress, relative humidity and partial and interupted imbibition will be required to understand, and adequately model, germination in the field.

2.2 Materials and Methods

2.2.1 Seed Sampling

Seedlots of *E. delegatensis* from five different seed zones in Tasmania were obtained from Forestry Commission, Tasmania (FCT) seed stores. Each seedlot represents bulked seed collections from 10 to 50 trees of a particular provenance within the seed zone. Details of the exact provenance location are not recoverable. Collection and extraction methods are detailed in Lockett (1991).

The seedlots were selected to maximise diversity of genetic material about three axes. The first axis of variation selected was the sub-populations recognised by Boland and Dunn (1985). The second and third axes were based on average temperature and average annual precipitation of the seed zone. The latter two axes are used by the FCT to determine seed zone equivalence in scheduling seed for sowing operations (FCT 1989). The seedlot characteristics are summarised in Table 2.1. Location of seed zones are shown in Fig. 2.1. The M36 seedlot was tested experimentally to determine the form of the germination response curves to temperature, moisture and stratification conditions. The other seedlots were tested less intensively, and were used to gauge the likely variation of response within the species in Tasmania.

Experimental seed samples were obtained by repeated sub-sampling from the bulked seedlots using a seed trier (Anon. 1985). The seed was then heaped and divided repeatedly into halves, each half being mixed prior to the next division, until eight test samples were obtained. Each sample was then corrected to the desired experimental weight (accurate to ±0.0005 g or approximately one seed particle). Under each set of test conditions an attempt was made to obtain approximately 100 viable seeds in each test sample, with at least four replicates. Because of experimental limitations, however, fewer viable seeds per replicate were used in the water potential experiments (approximately 50 per test sample).

Table 2.1. Characteristics of seedlots used in experimentation.
The proportion of dormant seed is estimated by comparing the proportion that germinate at 20°C after 56 days stratification to the number that germinate without stratification. The temperature classes are from the FCT (1989) seed zones, and the sub-populations are the sub-regional groupings of Boland and Dunn (1985).

Seedlot	Proportion dormancy	Altitude class (m)	Temperature class	Rainfall class (mm)	Sub-popⁿ
L17	0	<300	mild	650-900	south-east
M32	0.52	300-700	cold	1200-1600	north-west
M36	0.50	300-700	cold	900-1200	central
M38	0.30	300-700	cold	650-900	south east
M50	0.62	300-700	very cold	>1600	north-west

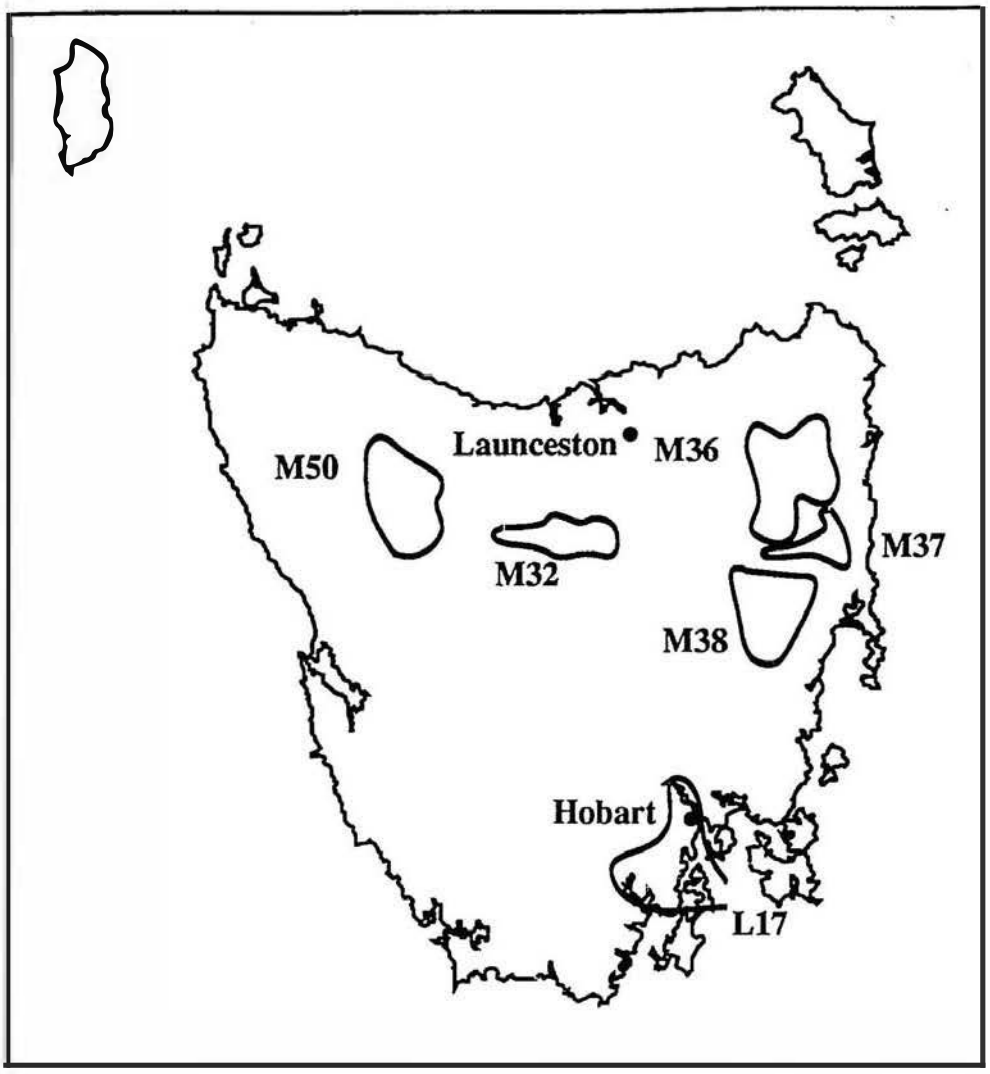


Fig. 2.1. Location of seed-zones from which seed samples used in this study were drawn (from Forestry Commission, Tasmania 1990). Seed-zone M37 is mentioned in Chapter 6.

2.2.2 Germination Test Conditions

A standard light source was used in all experiments, although the photoperiod varied between 12 and 18 h. Light of photon flux density between 100 and 200 μmol m^{-2} s^{-1}, and wavelength between 400 and 700 nm, was provided by a mixture of incandescent (approximately 20% of total wattage) and fluorescent sources. Although it was noted in Chapter 1 that the germination capacity of seed samples has been found to be higher in the dark than in the light (McLeod 1981) these experiments were conducted with a diurnal light-cycle, simulating the light regime that seeds would be subjected to when sown onto forestry coupes following logging operations. Some seeds might become buried under a thin soil layer and germinate in the dark, but observation has indicated that many seeds germinate when on the surface or only partly buried.

All temperature and stratification experiments were carried out in sealed plastic containers on filter paper over a towelling water reservoir. Constant temperatures were maintained in controlled environment chambers, accurate to within 0.5°C of that specified. Stratification was done at 5°C, under similar conditions. Seed samples were watered regularly to maintain a water supply in the towelling reservoir, but not to the point where free water was visible on the surface of the filter paper.

Experiments examining the effects of soil water potential on germination were conducted by suspending cones of dialysis tubing, filled with approximately 30 cc of soil, in solutions of known water potential and allowing the soil water matric potential to come to equilibrium with the solution osmotic potential. This technique has been used successfully by others to generate matric potentials in soil samples (Kaufmann 1969; Kaufmann and Ross 1970; Waldron and Manbeian 1970; Sharma 1973). The cones were made by folding a 10 cm disk cut from wide dialysis tubing. These were filled with fine sand of particle size between 63 - 211 mm, attached to wire loops with plastic paper-clips and suspended five to a rack in approximately 1.5 L of osmoticum, such that the solution level was approximately 5 mm below the soil surface (Plate 2.1). Soil samples were allowed to equilibrate for 14 days prior to the seeds being spread on the surface. Solution osmotic concentrations were controlled by the addition of polyethylene glycol 6000 MW, the concentration determined by the algorithm of Michel and Kaufmann (1973). Soil water potentials equal to or below -0.05 MPa were tested with a Microvolt Dewpoint meter (Wescor 33RT) and found to

Plate 2.1. Experimental apparatus used to apply soil matric water potentials in germination experiments.

Table 2.2. The set of test conditions used in examining the germination of *E. delegatensis*
The M36 seedlot was subject to all test conditions, other seedlots only to those test conditions in bold type. The effect of the underlined water potentials on the germination of the M36 seedlot were tested at 12.5°, 17.5°, 20°, 22.5° and 25°C.

Temperatures:	Seeds were germinated at 2, 5, **7.5**,**12.5**, 15, **17.5**, 20, **22.5**, 25°C constant temperature and 10/20 (14 hour photoperiod) and 15/20°C (18 hour photoperiod) alternating temperature.
Stratification:	Seeds stratified for **0**, **7** , **14**, **28** and **56** days were tested at all germination temperatures.
Strengthened dormancy experiments:	
	Seeds were stratified for 0 (imbibed 24 hours at 20°C), 7, 14, 28 and 56 days and then held at 35°C for 24 hours and then germinated at 20°C.
	Seeds imbibed for 24 hours were exposed to 25, 30 and 35°C for 24 hours and then germinated at 20°C.
	Seeds imbibed for 24 hours at 20°C were subjected to 35°C for 8 hours and then stratified for 0, 14, 28 and 56 days before being transferred to 20°C to germinate.
Water potential:	**0**, -0.001, -0.0025 -0.005, -0.0075, -**0.01**,-0.025, -0.05, -0.075, -**0.1**, -**0.25**, -**0.5**, -**1** at 20°C .
wetting and drying:	Seeds were dried for 24 hours after 24, 48, 60, 80, 120, 140 and 160 hours imbibition.
	Seeds were dried for 24, 48, 72 and 168 hours after 48 hours imbibition.
	Seeds were wet and dried for 1, 2 and 3 cycles, with 24 hours wet between cycles, and with a total interval dry of 72 hours.
imbibition :	Seeds were imbibed at 0 MPa at 50% and 100% RH.
	Seeds were imbibed at 20°C in solutions of 0, -0.05,-0.1, -0.5,-1, -2 and -3 MPa.
	Seeds imbibed at 0, -1, -2 and -3 MPa were transferred to solutions of 0 MPa after 7, 14 and 28 days.
	Seeds were imbibed at 0 MPa at 5°, 12.5°, 17.5°, 20° and 25°C.

be close to the predicted value. Values above this were beyond the sensitivity of the machine and need to be treated with caution.

Experiments in which wetting and drying conditions were applied were conducted in closed 90 mm diameter petri dishes with three layers of Whatman grade 182 filter paper at 20°C. Water was added so that no free water remained on the surface, but such that tilting the dish resulted in water draining to one side. Additional water was added as necessary to maintain this level of water availability. Five replicates each of 20 seeds were used. Squash testing following experimentation revealed that in all cases viable seeds had been selected. Seeds were dried by transferring to dry filter papers and leaving uncovered in a laboratory in which the ambient humidity varied between 40%

and 50% RH. After 2 hours, seeds in such conditions had returned to their air dried weight. Seed weights taken during the course of germination were obtained by first blotting the seeds dry with tissue paper and then weighing.

Imbibition experiments were carried out in petri dishes as previously specified. Constant temperatures were obtained in the seed germination cabinets previously described. Moisture stresses were applied by imbibing in solutions of polyethylene glycol described above. All treatments were carried out in sealed petri dishes (100% RH) except for the 50% relative humidity treatment, for which the petri dishes were left open in a room in which the ambient humidity was approximately 50%. To prevent changes in solution water potential, filter papers and solutions were replaced each day.

The total set of test conditions used in the experiments is given in Table 2.2.

2.2.3 Measurement of Germination Performance

Monitoring of seeds was performed daily until germination fell to a level where it was considered that less frequent scoring would be adequate. In cases where germination was particularly slow (e.g. at 5°C) scoring was spaced at longer intervals. Seeds were considered to have germinated as soon as the embryo ruptured the testa. Experiments were scored until germination ceased, usually one week without germination. The mean number of germinants from the treatment combination that gave the highest number of germinants was assigned a germination capacity of 100%. Other test conditions that resulted in lesser germination were assigned germination capacities relative to this value. The use of germination capacity in this thesis is, therefore, a relative rather than an absolute measure of germination capacity.

The rate of germination was estimated from the reciprocal of the time taken to reach 50% of the final cumulative germination, $t50$, under the test conditions following the commencement of imbibition. Being a rate measure based on the median, $t50$, unlike many other rate measures [e.g. germination energy index (Grose 1963) or the mean time to germination (Kotowski 1926)], is relatively insensitive to long tailed or slightly skewed distributions (Nichols and Heydecker 1968; Orchard 1977). Cumulative germination curves were generally sigmoidal in shape and the variation in percentage germination with increasing time was approximated to the normal frequency distribution (Finney 1952). A probit transformation, which transforms percentage germination values to normal

deviates, was used to linearize the relationship between cumulative germination and the logarithm of time. The *t50* values in this study were calculated from the regression of probit-transformed germination times against the natural logarithm of elapsed time.

2.3 Results

2.3.1 Temperature and Stratification

The effect of temperature on the rate of germination of the M36 seedlot is shown in Fig. 2.2. The rate of germination increased with temperature to an optimum and then declined. This optimum was more sharply defined for seedlots subjected to longer periods of stratification. Irrespective of the duration of stratification the optimum temperature for germination rate was approximately 20°C. No germination occurred at 2°C after 120 days and, although seeds may have eventually germinated at this temperature, I assume that the low temperature threshold for germination lies between 2 and 5°C. The upper temperature threshold was not explored, but is higher than 25°C. Alternating temperatures did not affect the rate of germination, and for comparison are shown in Fig. 2.2 converted to average daily temperatures of 16° and 19°C. Stratification increased the rate of germination at all temperatures, with the increase in rate being approximately linear with stratification period (Fig. 2.3). Germination rate increased with temperature for all seedlots tested in the range 5° to 17.5°C and seeds within a seedlot germinated at almost equal rates at 17.5°, 20° and 22.5°C (Fig. 2.4). The L17 seedlot germinated more rapidly at all temperatures tested above 5°C. The M50 seedlot more slowly than other seedlots at high temperatures. The germination rate of all seedlots, except for the M50 seedlot, was significantly reduced at temperatures of 12.5°C. All seedlots germinated very slowly at 7.5°C.

Germination capacity was also influenced by temperature and duration of stratification period (Fig. 2.5). Complete germination of the viable seed proportion occurred only after stratification. Non-stratified seed germinated almost equally well at temperatures between 15° and 20°C, but germinated poorly outside this range, except when temperatures were low enough to stratify seed. Consequently, the response curve of non-stratified seed to temperature exhibits a bimodal form. As the stratification period was increased, the range of temperatures over which a high proportion of the seed germinated increased, so that after 56 days stratification most of the viable seed fraction germinated at all

temperatures tested between 7.5 and 25°C. The temperature at which maximum germination capacity was observed appeared to increase with stratification period. With no stratification germination capacity was highest at 15°C, increasing to 20°C after 56 days stratification. Unlike germination rates, germination capacities under regimes of alternating temperature are not equivalent to what would be expected from interpolation from constant temperature regimes (Fig. 2.5). Germination under the 20/10°C (equivalent day degrees to constant 16°C) temperature regime was consistently slightly lower than would be expected from linear interpolation and the 20/15°C regime (equivalent to constant 19°C) is consistently slightly higher.

Considerable inter-seedlot variation was exhibited in germination capacity, both in the proportion of seed that was dormant and in the response profile to germination temperature. The initial dormancy in the seedlots varied from near 0% to 60% of seeds (Fig. 2.6). The release from dormancy by stratification differed between seedlots. Except for the M50 seedlot only a slight gain in germination capacity was made by stratifying for longer than 28 days. The germination capacity of the M50 seedlot was unaffected by 14 days stratification but increased greatly following 28 and 56 days stratification. After 56 days stratification, many viable seeds still remained ungerminated (as suggested by squash testing: the 100% figure on the graphs indicates the proportion of the total amount observed to germinate relative to the number observed following 56 days stratification) suggesting that longer stratification would improve the germination capacity of the M50 seedlot further. By contrast the germination capacity of L17, which was already very high without stratification, was not improved.

The response of the four seedlots from the medium altitude range to germination temperature was similar (Fig. 2.7). The low altitude seedlot, L17, however, was quite different in its temperature-response profile germinating equally well at all tested temperatures below 25°C. In common with other tested seedlots the L17 seedlot has an optimum temperature for germination below 22.5°C. The high germination capacities displayed at 5°C by the other seedlots can be attributed to the removal of dormancy by the stratification effect at this temperature.

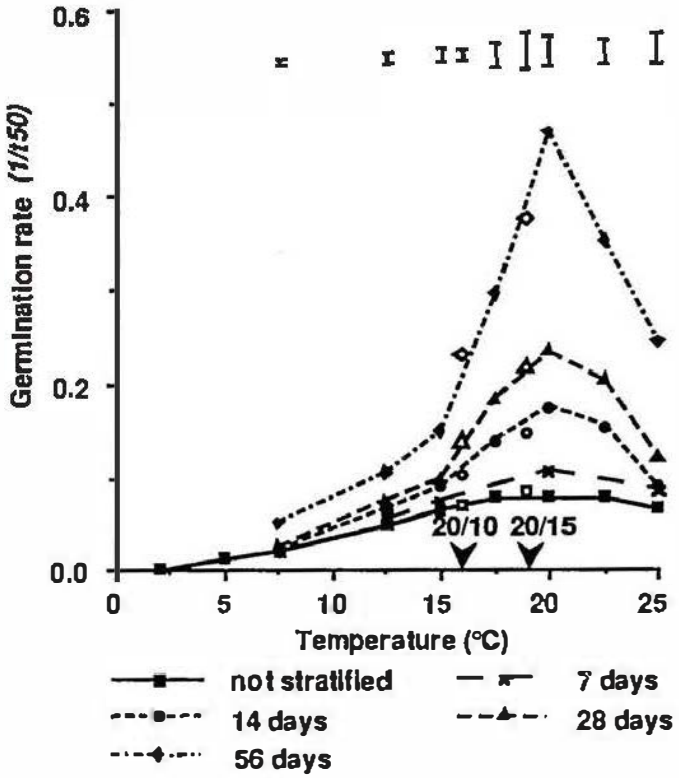


Fig. 2.2. The effect of temperature on the germination rate of M36 seed. Hollow symbols indicate the thermal sum equivalents of alternating temperature regimes. Error bars are the least significant difference for multiple comparison.

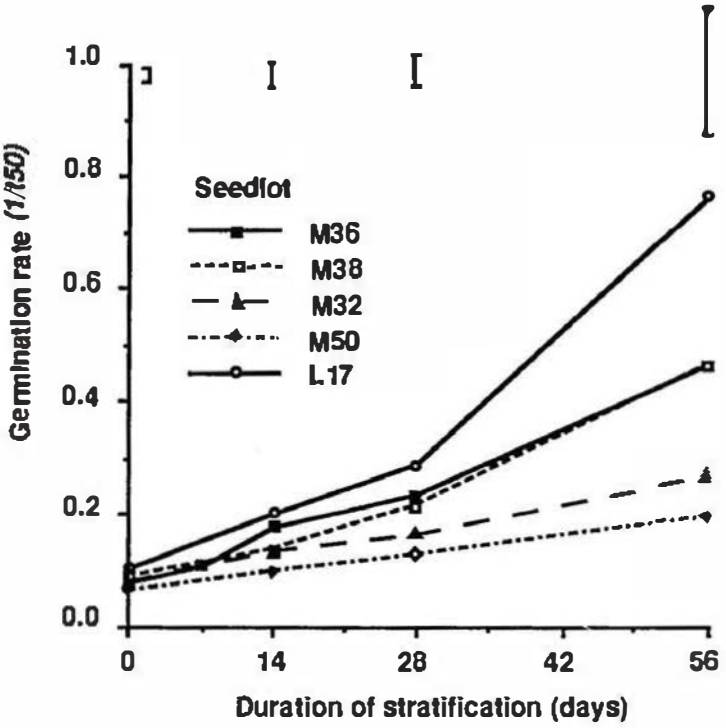


Fig. 2.3. Effect of the duration of stratification on germination rate at 20°C. Error bars are the least significant difference for multiple comparison of means.

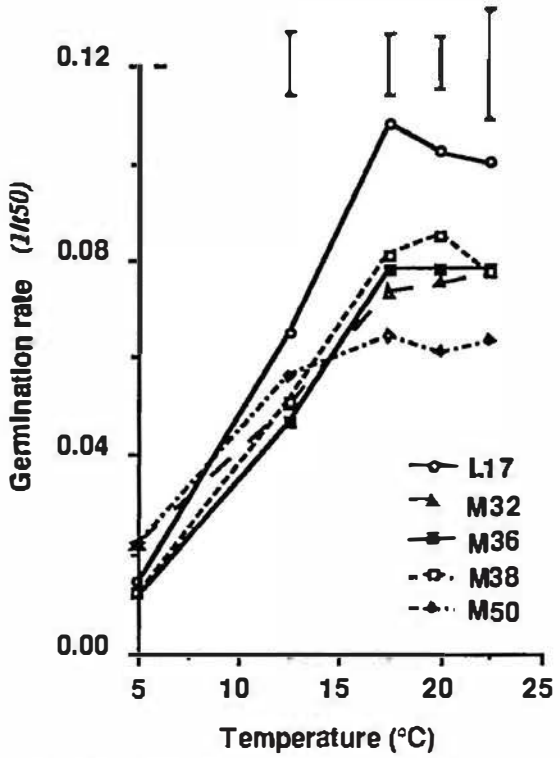


Fig. 2.4. Variation in germination rate response to temperature. Error bars are the least significant difference for multiple comparison.

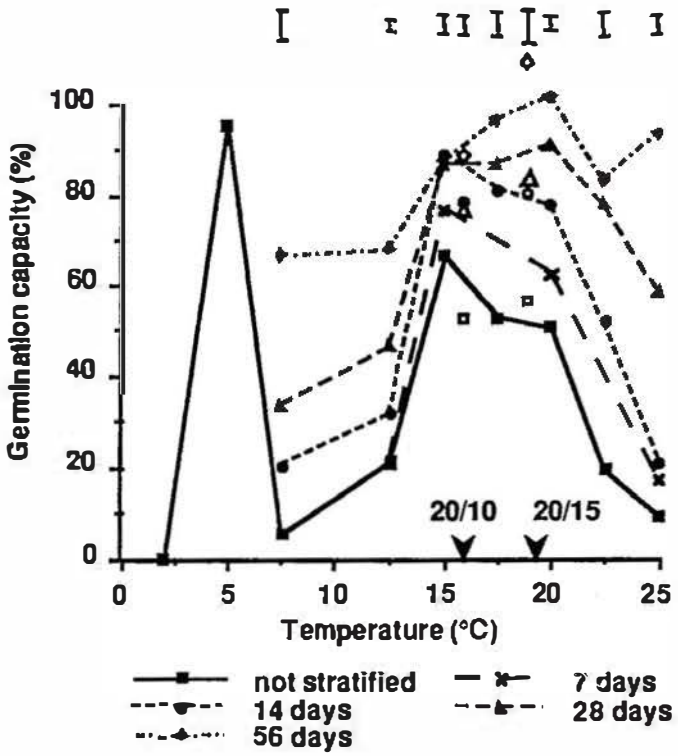


Fig. 2.5. Effect of stratification on germination capacity response of M36 seed to temperature. Hollow symbols indicate the daily mean equivalent of alternating temperature regimes. Error bars are the least significant difference for multiple comparison.

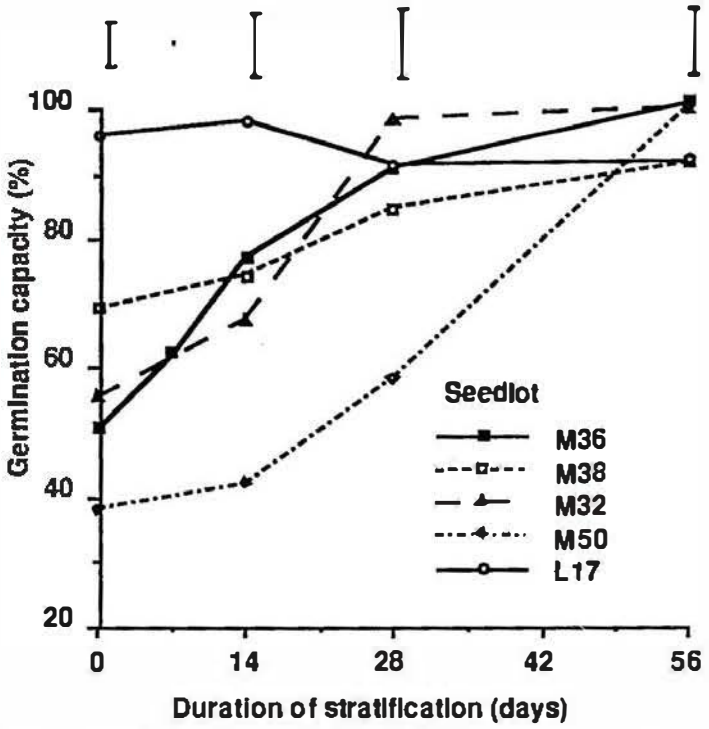


Fig. 2.6. Seedlot variation in dormancy and dormancy relief by stratification. Error bars are the Tukey-Kramer least significant distance for multiple comparison.

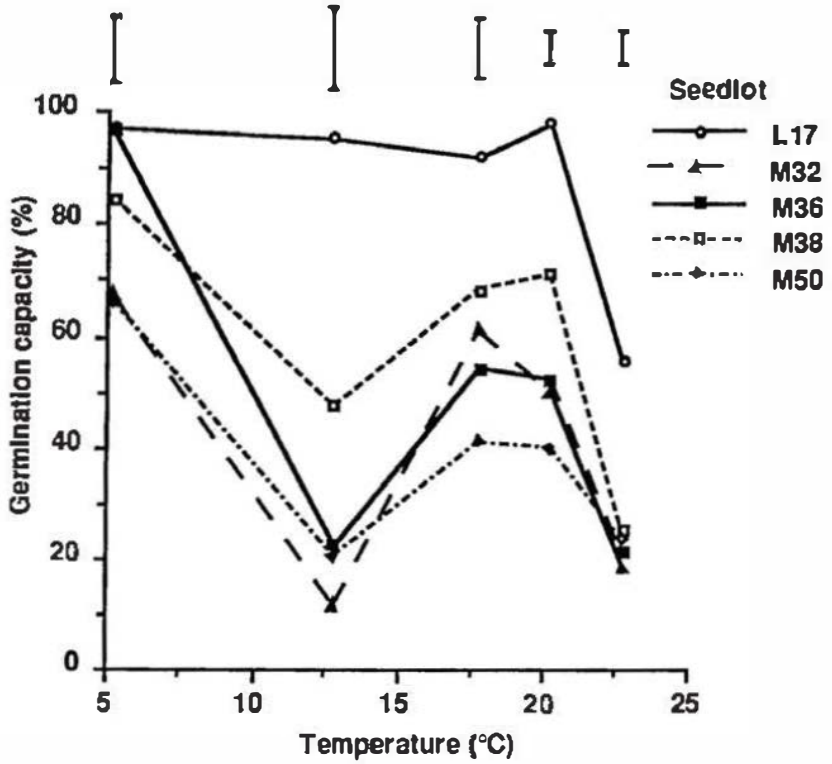


Fig. 2.7. Seedlot variation in germination capacity response to temperature. Error bars are the Tukey-Kramer least significant difference for multiple comparison.

2.3.2 Strengthening of Dormancy

All test samples exposed to high temperatures showed at least a slight decline in germination capacity (Fig. 2.8). Primary dormancy, however, was not easily strengthened nor secondary dormancy easily induced. The most severe treatment, exposure to 35°C for 48 h, caused a reduction in germination capacity from 50.8 ± 2.0% to 39.8 ± 1.6%. Shorter durations of exposure caused lesser reductions in germination capacity, with exposure for 8 and 24 hours resulting in germination capacities of 43.0 ± 1.9% and 45.0 ± 2.2% respectively. The effect of exposure to 30°C and 35°C for 24 hours were comparable (43.5 ± 1.2% and 45 ± 2.1% germination capacity respectively), however exposure to 25°C caused only a negligible reduction in germination capacity (49.8 ± 0.6%). The duration for which seeds were stratified prior to exposure to high temperature did not affect germination capacity relative to control seed samples. Stratification following exposure however suggests that the more dormant fractions of the seed population are more susceptible to strengthening of dormancy. Only small differences between control seed samples and seed samples exposed to 35°C for 24 h were apparent if seed samples were subsequently stratified for 14 days or less, however seed samples subsequently stratified for 28 and 56 days showed large relative decreases in germination capacity. That is, although the germination capacity of seed exposed to 35°C improved with stratification, it still did not match the improvement in germination capacity of the control seedlot with stratification (Fig. 2.8).

2.3.3 Soil Matric Potential

Germination capacity was relatively unaffected by soil matric potentials above -0.1 MPa for all seedlots except the L17 seedlot which remained unaffected by matric potentials of -0.25 MPa, and still displayed moderate germination at -0.5 MPa (Fig. 2.9). No seeds from any seedlot were able to germinate at -1 MPa. The response of germination capacity to the interaction of temperature and water potential is given in Fig. 2.10. Analysis (GLM on SAS (SAS 1989)) indicates a significant interaction ($p<0.01$) between temperature and matric potential (Table 2.3a.) with seeds germinating at, or near, their temperature optimum (17.5 and 20°C) being more sensitive to reductions in soil moisture above -0.1 MPa than seeds germinating at either sub-optimal (12.5°C) or super-optimal (22.5 and 25°C) temperatures.

The response of germination rate to soil matric potential was similar to the response of germination capacity. The response of the germination rate of all seedlots to soil matric potential was similar. Seedlot germination rates appeared to be relatively insensitive to soil matric potentials greater than -0.1 MPa, but dropped considerably at lower matric potentials, being reduced by 25-50% by -0.5 MPa, and with germination unable to proceed at -1 MPa (Fig. 2.11). Analysis (GLM on SAS (SAS 1989)) showed no interaction between the effects of temperature and soil matric potential on the rate of germination ($P>0.47$) (Table 2.3b; Fig. 2.12).

Table 2.3. Analysis of variance table for temperature and matric potential effects.

a. Germination capacity

Source	DF	Sum of Squares	Mean Square	*F* Value	*p*
temperature	4	1.3769	0.3442	24.15	0.0001
matric potential	3	2.2490	0.7497	52.6	0.0001
interaction	12	0.5018	0.0418	2.93	0.0012
error	140	1.9954	0.0142		
b. Germination rate					
temperature	4	0.0982	0.0245	26.01	0.0001
matric potential	3	0.0762	0.0254	26.90	0.0001
interaction	12	0.0110	0.0009	0.97	0.4787
error	140	0.1322	0.0009		

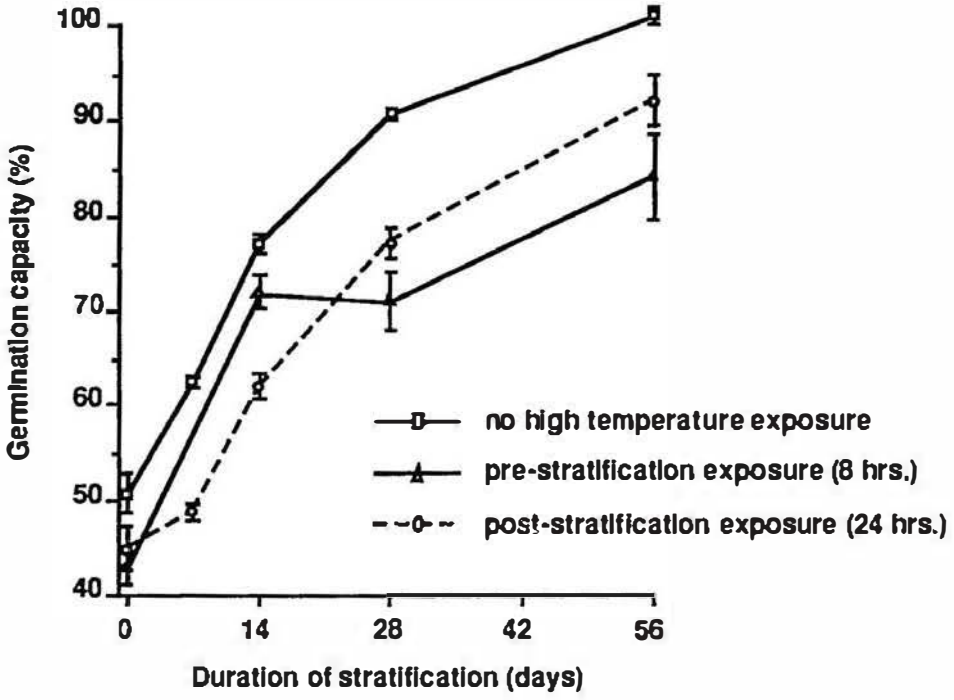


Fig. 2.8. Effect of exposure to 35°C prior to and following stratification on germination capacity of M36 seed germinatec subsequently at 20°C. Error bars are the 95% confidence interval of the mean.

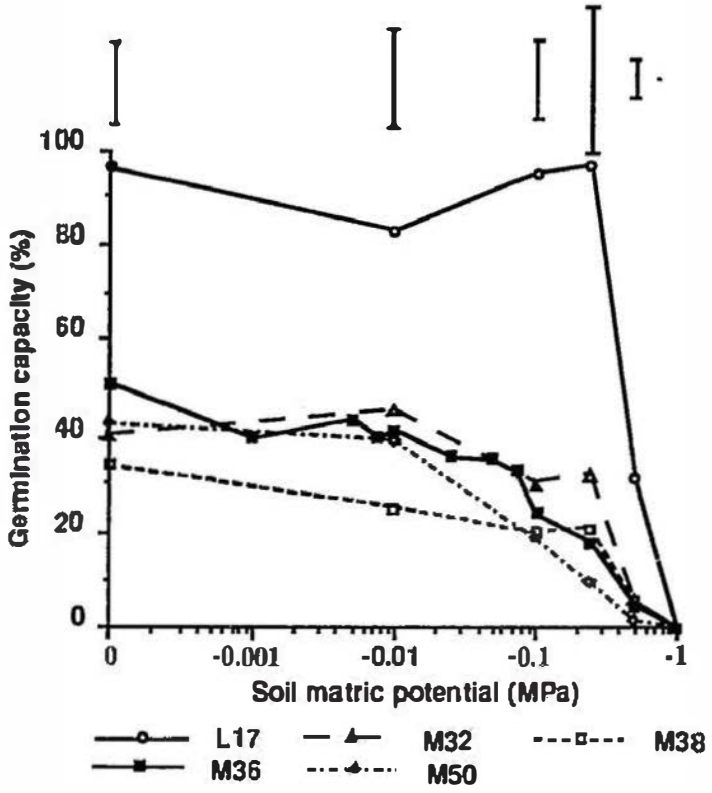


Fig. 2.9. Seedlot variation in germination capacity response to soil matric stress. Error bars are the Tukey-Kramer least significant difference for multiple comparisons. Note the x-axis uses a logarithmic scale.

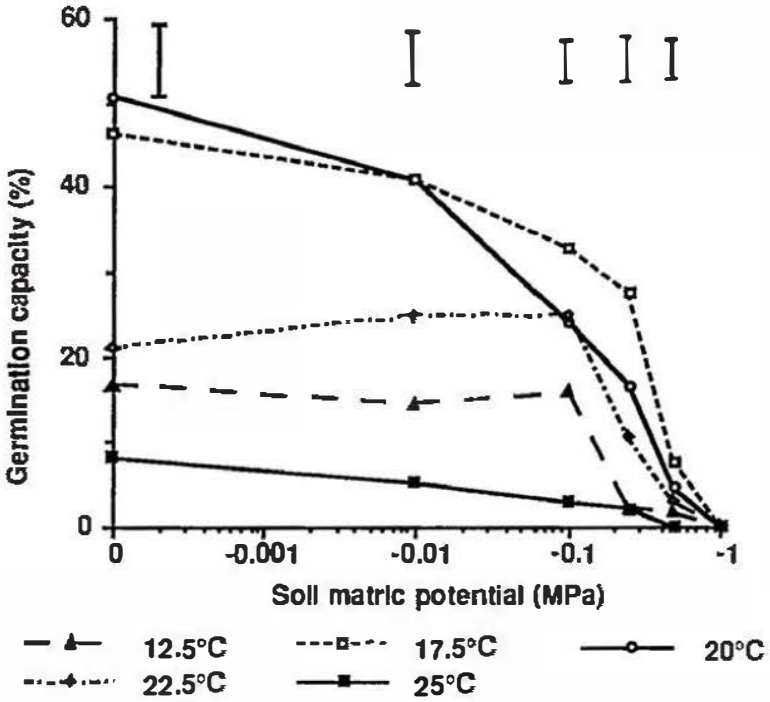


Fig. 2.10. The interaction of temperature and soil matric potential on germination capacity of the M36 seedlot. Error bars are the Tukey-Kramer least significant difference for multiple comparison. Note that the x-axis is expressed on a logarithmic scale.

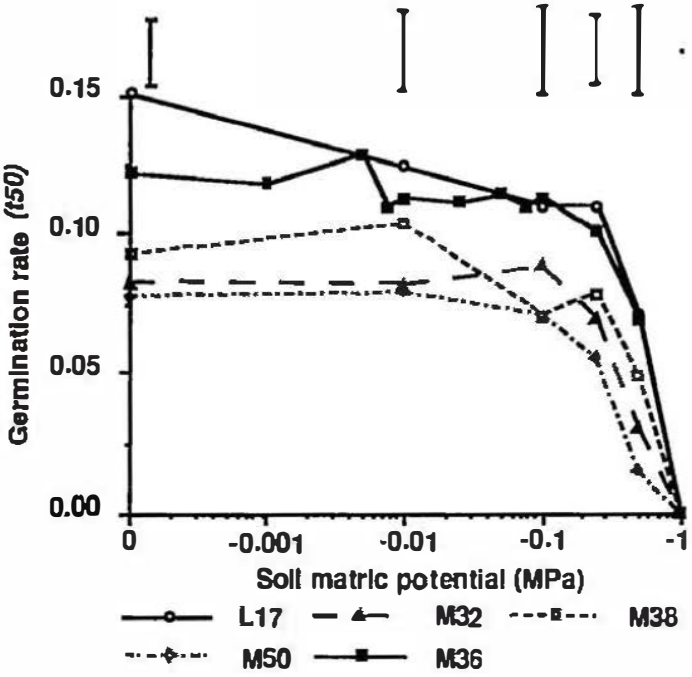


Fig. 2.11. Seedlot variation in germination rate response to soil matric potential. The error bars are the Tukey-Kramer least significant difference for multiple comparison. Note that the x-axis is expressed on a logarithmic scale.

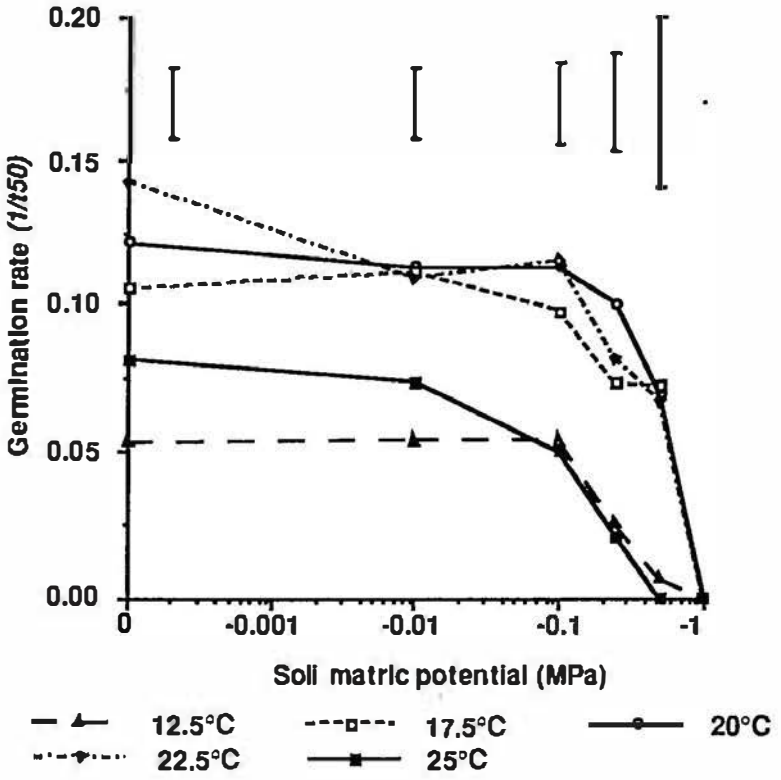


Fig. 2.12. The interaction of temperature and soil matric potential on the gennination rate of seed from the M36 seed zone. Error bars are the Tukey-Kramer least significant difference for multiple comparison Note that the x-axis is expressed on a logarithmic scale.

2.3.4 Imbibition and Pre-imbibing of Seeds

Seeds stored, or 'primed', in osmotic solutions down to -2 MPa for 7 days germinated more rapidly when transferred to solutions at 0 MPa than seeds that had not been pre-imbibed. Storage of seeds at -3 MPa did not increase germination rate. This increase in germination rate appeared to be due principally to a change in the time to the commencement of germination, but in the case of seeds held at -0.5 MPa also more synchronous germination (Fig. 2.13). Without priming seeds commenced germination after 7 days, and had completed 50% of germination after 9.1 days with a mean time to germination of 13.2 days. Seeds held initially at -0.5 MPa for 7 days commenced germination within one day of transfer to a non-water limiting environment and had completed 50% of total germination after 1.5 days with a mean time to germination of 4.0 days. Seed samples primed at -1,-2 and -3 MPa all had reduced times to commencement of germination relative to non-primed seeds, 3, 5 and 4 days respectively. However, the spread of germination times once germination had commenced was similar or greater than that of non-primed seed samples. Holding seeds at these water potentials for longer than 7 days did not appear to increase the germination rate. Fig. 2.14 shows cumulative germination after holding seeds at -2 MPa for 7, 14 and 28 days prior to transferring to a solution of 0 MPa. Unlike the effects on germination rate, storage at these water potentials did not result in a consistent effect on germination capacity.

Seeds imbibed without interruption at 20°C with humidity kept at 100% began to germinate after approximately 100 h, and completed germination after 400 h. Water uptake at 20°C was rapid for the first 24 h, until seeds obtained a relative water content (i.e. 100*[seed wet weight-seed dry weight]/air dried weight) of approximately 40 per cent (Fig. 2.15). This was followed by a period of relatively slow water uptake, until relative water content once again increased rapidly as emergence commenced. While the first radicles emerged after 100 h, the first seed coats were observed to be ruptured after 60 h. Imbibition was noticeably triphasic, an initial rapid uptake of water, a relatively long period of minimal water uptake, and a third period of rapid increase in relative water content associated with the emergence of the radicle and vegetative growth. Emergence began approximately 80 hours after seeds finished rapid water uptake and achieved a relative water content of 40%. Seeds imbibed at 50% relative humidity increased in weight more slowly than those imbibed at 100% relative humidity. Water uptake was rapid until seeds had a relative water content of approximately 35%, and then continued to rise more slowly. Once again

germination commenced approximately 80 hours after seeds achieved a relative water content of 40%. It is possible that seeds germinating in the absence of soil water stress require to be held at a relative water content of 40% for at least 80 h to commence germination.

The rate of imbibition was also affected by the temperature at which imbibition occurred (Fig. 2.16). At 5°C, it took approximately 120 hours for seeds to obtain a relative water content of 40%. As the ambient temperature was increased initial rate of water uptake also increased so that at 22.5 °C the relative water content was 40% after 12 h.

The rate of uptake of water was unaffected by the osmotic potential of the solution in which they were imbibed within the range 0 to -0.5 MPa. Water uptake at -1,-2 and -3 MPa, however, was markedly impeded and seeds had still failed to obtain a 40% relative water content increase after 672 h (Fig. 2.17).

2.3.5 Wetting and Drying Cycles

Drying of imbibed seeds within the first 24 h had no effect, and drying within the first 48 h had only a small effect on germination capacity (Fig. 2.18). Dehydration at a later stage caused a reduction in germination capacity; the later the dehydration of the seed samples the greater was the impact on germination capacity. Once most of the seeds in the sample had ruptured their seed coats and radicle emergence had begun the effect of dehydration on germination capacity was severe. Similarly, dehydration of seeds decreased the rate of germination, even if the time prior to dehydration was discounted, the reduction being more pronounced the longer the time that had elapsed since the commencement of imbibition. Neither cycles of wetting and drying (Fig. 2.19) nor the length for which seeds remain dry following dehydration (Fig. 2.20) affected the germination capacity or germination rate above that caused by the timing of the last drying.

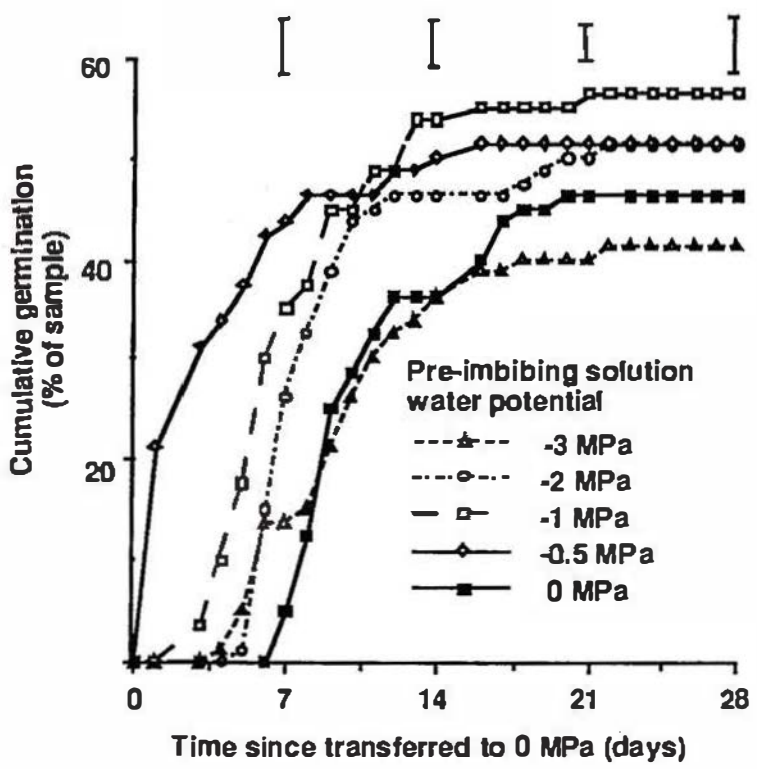


Fig. 2.13. Effect of pre-imbibing of seeds for 7 days in solutions of differing osmotic potential prior to transfer to a solution of 0 MPa on cumulative germination. Error bars are the Tukey Kramer least significant difference for multiple comparison.

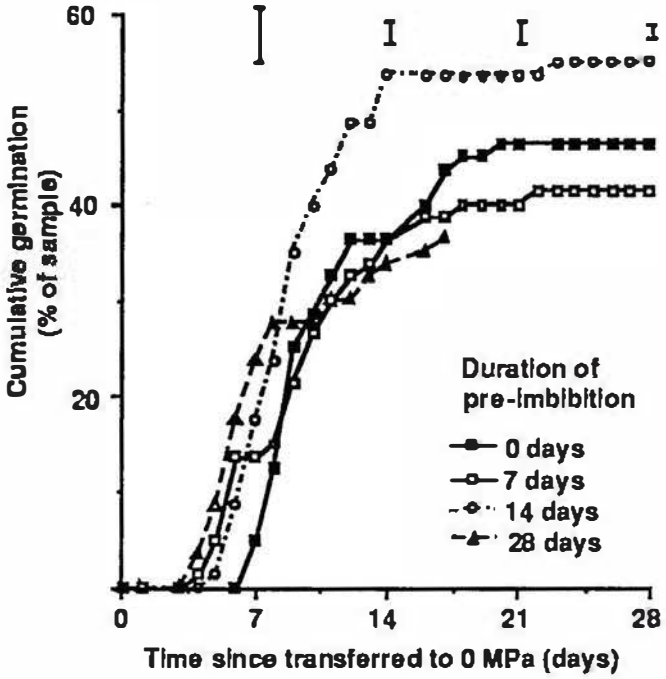


Fig. 2.14. Effect of duration of pre-imbibition at -2MPa on the cumulative germination once transferred to a solution of 0 MPa. Bars are the Tukey-Kramer least significant difference fo multiple comparison.

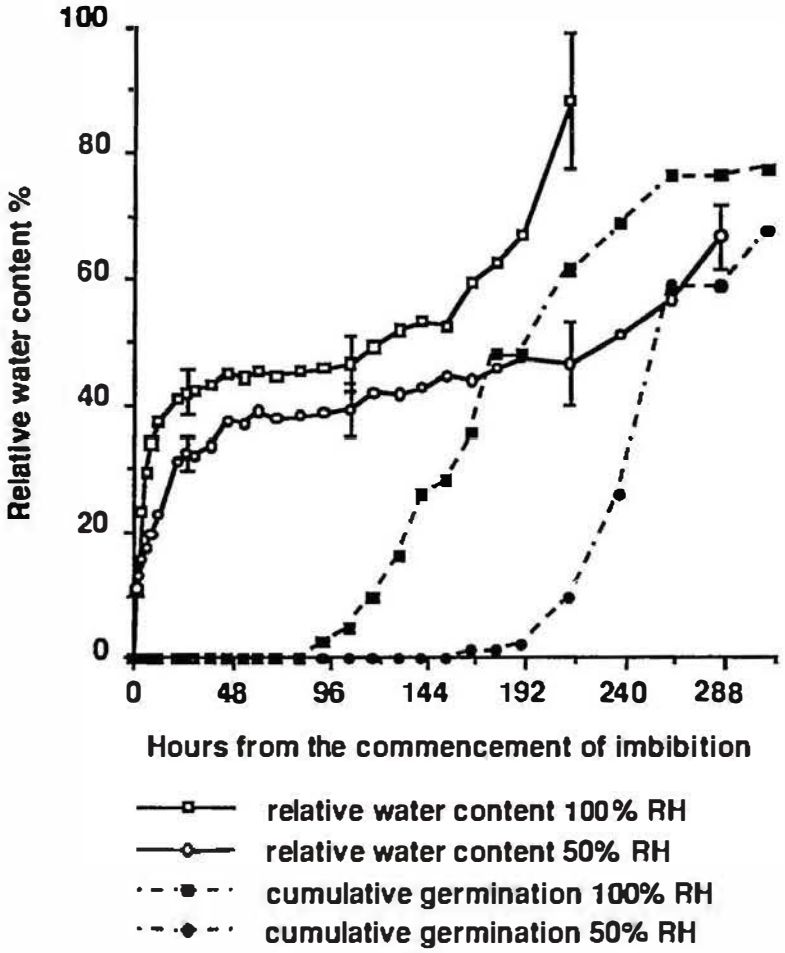


Fig. 2.15. The effect of relative humidity on the imbibition rate and time to commencement of germination. Error bars are the 95% confidence interval of the mean.

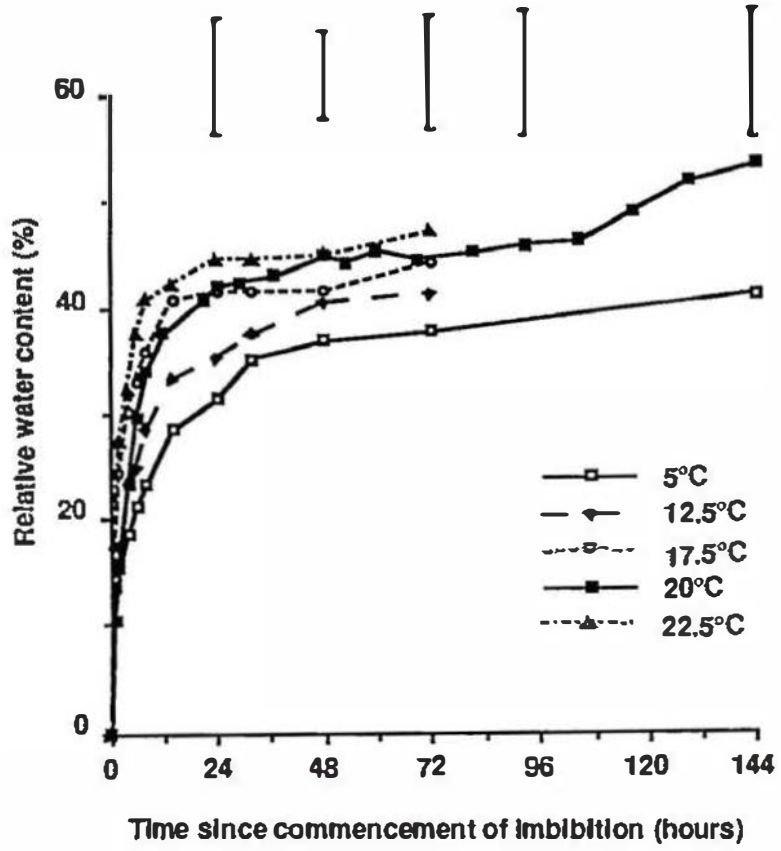


Fig. 2.16. The effect of temperature on the imbibition rate of seeds. Error bars are the Tukey-Kramer least significant difference formultiple comparison.

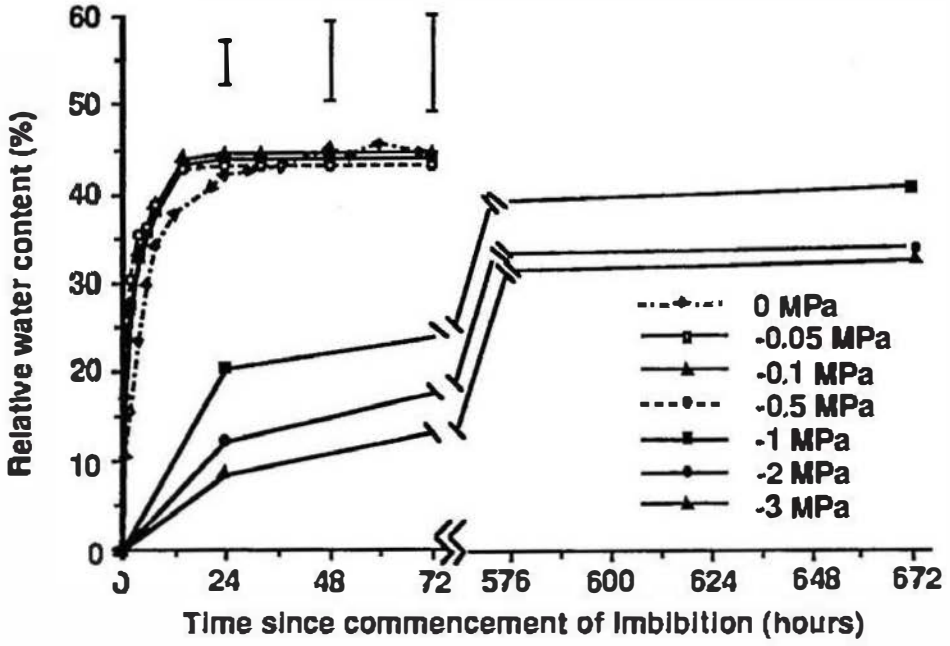


Fig. 2.17. The effect of osmotic potential on the imbibition rate of seeds at 20°C, M36 provenance. Error bars are the Tukey-Kramer least significant difference for multiple comparison.

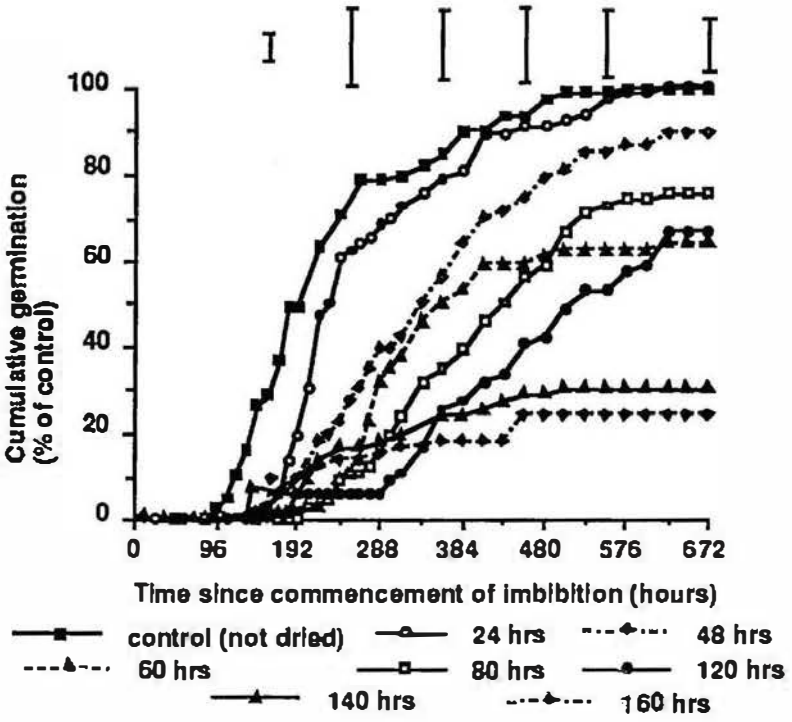


Fig. 2.18. Effect of timing of dessication following commencement of imbibition on cumulative germination. Error bars are the Tukey-Kramer least significant difference for multiple comparison.

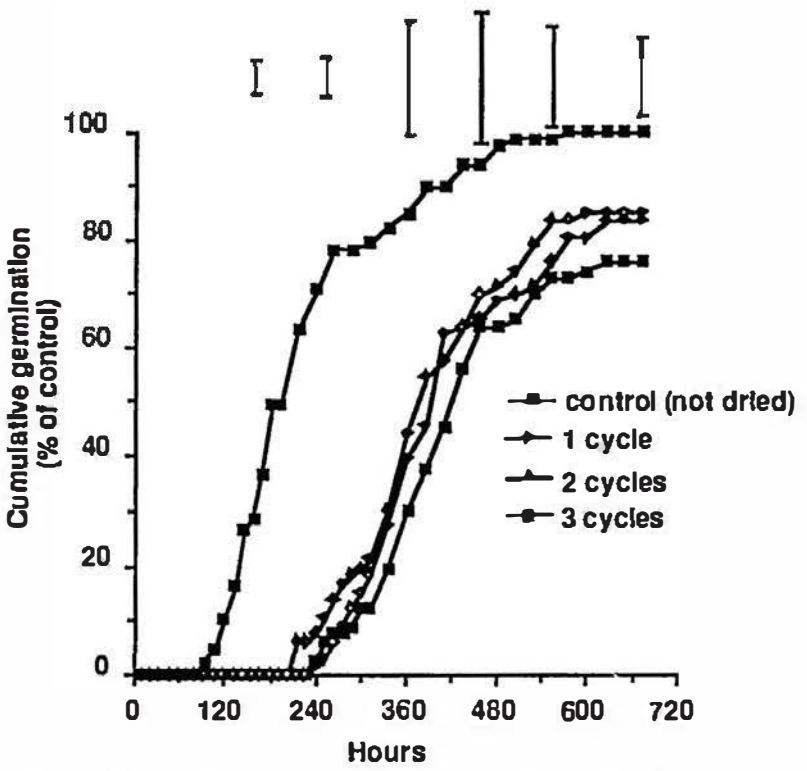


Fig. 2.19. Effect of cycles of wetting and drying on cumulative germination. 1 cycle=48 h wet, 72 h dry, and then wet; 2 cycles=w24-d48-w24-d24-wet; 3 cycles=w24-d24-w12-d24-w12-d24-w. Error bars are the Tukey Kramer least significant distance for multiple comparison.

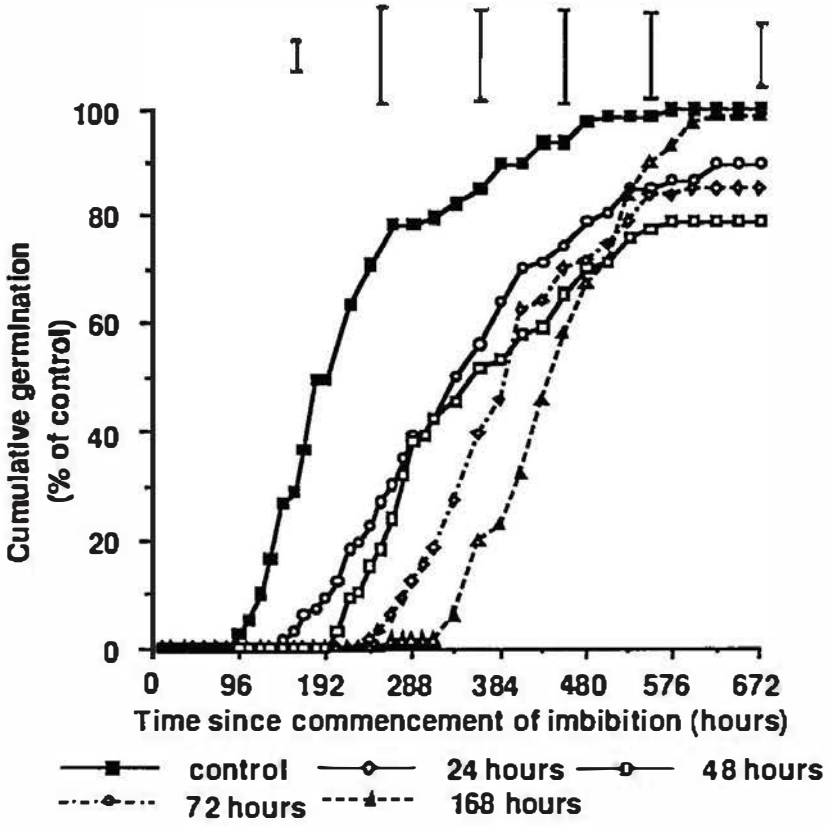


Fig. 2.20. Effect of the duration for which seeds are dried following 48 hours imbibition on cumulative germination. The x-axis displays total time elapsed since the commencement of imbibition including the time for which seeds were held dry following interruption to the imbibition/germination process The error bars are the Tukey-Kramer least significant difference for multiple comparison.

2.4 Discussion

2.4.1 Germination Responses

The response of rate of germination and germination capacity to temperature found in this study are comparable in many regards to past studies by Grose (Grose 1957*b*; 1963). Germination capacity displayed a distinct temperature optimum in the range 17.5° to 22.5°C, with this optimum being less pronounced after stratification, and the lower temperature threshold was approximately 2°C. However, some notable differences were observed.

Whereas Grose (1963) found that the germination capacity of Victorian seedlots exposed to temperatures of 25°C or higher for 8 hours was adversely affected, the germination capacity of Tasmanian seedlots examined in this study appeared insensitive to comparatively harsh treatments. The effects of exposure to high temperature become most apparent following stratification (Fig. 2.8). It appears that possibly only the most dormant seeds, that is those which would be expected to germinate only after a long period of stratification, were susceptible to having dormancy strengthened by the test conditions. By contrast, stratification prior to high temperature exposure, presumably by reducing the depth of dormancy of the population, reduces the susceptibility of the seeds to strengthening of dormancy. The seedlot used by Grose (1963) in his experimentation was 70% dormant. It is possible that the lower initial proportion of dormancy of the populations tested in this work makes them less susceptible to the strengthening of dormancy. Tasmanian populations of *E. delegatensis* with lower inherent dormancy than their Victorian counterparts (54% compared to 79%) may not be as susceptible to the induction of high temperature dormancy. Cunningham (1960) showed that *E. regnans* seed in the surface layers of soil may have a moisture content as high as 40-50% when their temperature is 35°C, but that seeds are unlikely to have moisture contents at which they are metabolically active at higher temperatures. Conditions which favour the strengthening of dormancy in the field may therefore not persist for long, perhaps only for short periods after rain in summer or autumn. Given the relative resilience of the tested Tasmanian seedlot to strengthening of dormancy, it is possible that strengthening of dormancy is not a significant factor for Tasmanian populations of *E. delegatensis*.

Also, contrary to previous work with the species (Grose 1957*b*; 1963; 1965), a temperature optimum for rate of germination was observed (Fig. 2.2). This is despite the highest temperature tested being lower than that of Grose (1963).

This difference may partly result from the different rate measures, germination energy index (GEI) and the reciprocal of time to 50% germination (*t50*), used in the respective studies. When the germination energy indices are calculated for this study's responses, only a slight, and non-significant, decline in rate of germination above the optimum temperature is observed. The GEI effectively integrates the area under the germination curve and takes it as a proportion of the area as defined by the product of the time to maximum germination and of the germination capacity. By increasing the ratio of these areas, long-tailed or positively skewed distributions reduce the sensitivity of the GEI to changes in germination rate. The *t50* measure, which takes the average slope to what is normally the steepest part of the cumulative germination curve, is reasonably robust in this rega d. The distribution of germination times of *E. delegatensis* at higher temperatures appea s to be particularly skewed (this study and MacLeod 1981). The presence of a temperature optimum above and below which the rate of germination declines has been noted for many species (Bewley and Black 1982). It seems likely that there is a decline in rate of germination above the temperature optimum, and the absence of this effect in the previous study is a result of the analysis method.

The decline in rate of germination with decreasing ambient temperature (Fig. 2.2), results, in part, from the decline in imbibition rate with temperature (Fig. 2.16). Such temperature dependency of water uptake has been noted in the imbibition of a number of plant seeds (Keller and Bleak 1970; Blacklow 1972) and it is the initial rapid water uptake phase associated with the wetting of the seed that is most sensitive to temperature, the slower second stage of water uptake associated with metabolic processes being comparatively insensitive to temperature (Dewez 1964). This variation in imbibition time with temperature, however, is relativly minor when compared to the total time until emergence begins. Many aspects of the germination process such as enzyme activity (Labouriau 1977; 1979), changes to membrane properties (Mayer 1986) and changes in testa permeability (Ivens 1983) are affected by temperature. The obser ed rate of germination will be the net effect of all these processes each of which may differ in temperature optima.

The rate of germination of seeds increased almost linearly with duration of stratification (Fig. 2.3), with the slight curvature of the relationship for the fastest germinating seedlots due to the commencement of germination of an increasing proportion of the seed population during stratification temperature with time. This linea relationship suggests that the increase in rate of germination after

stratification is a result of progress towards germination during stratification. The increase in germination capacity over a wide range of temperatures following stratification suggests that stratification also reduces the sensitivity of seeds to conditions that increase the proportion of dormancy in untreated seed. Seeds can be considered to exist in three states: they can be dormant and hence unable to germinate under any set of conditions without some period of prior stratification; they may be non-dormant and able to germinate under a range of conditions but be susceptible to becoming dormant; or they may be non-dormant and relatively impervious to the ambient temperature. Stratification may act to increase the rate of germination via an accrued 'thermal time' (*sensu* Wang 1960). It may increase the germination capacity of seed samples by increasing the proportion of seeds that are non-dormant as well as increasing the proportion of seeds that have progressed sufficiently far along the germination path to be relatively impervious to dormancy inducing factors.

The germination response to water stress in this study differs substantially from the work of Gibson and Bachelard (Bachelard 1985; Gibson and Bachelard 1986*a*, 1986*b*, 1987, 1988) who found that seed germination in a range of eucalypt species was affected by matric potentials as high as -0.003 MPa. Most of the experiments by these other workers were conducted directly on a sintered plate, a medium on which seed contact is poor and hence seeds would be presumed to be highly susceptible to any decline in moisture levels, however Bachelard (1985) applied tensions to a 1.5 cm deep (although the final depth was not determined) soil slurry on a ceramic plate . The average particle size of the soil was similar to that used in this experiment. The responses to imposed soil matric potential found in the current work (Fig. 2.9) were comparable to results found by workers examining the effect of water stress on germination rate and germination capacity using osmotic solutions to impose stress (Zohar *et al.* 1975; Edgar 1977). This work found that stresses of the order of -0.1 to -1.0 MPa were required to affect germination. These results are in accord with field observations in which *E. delegatensis* is observed to germinate under relatively dry conditions, certainly conditions far drier than -0.003 MPa, a situation that must only persist for short periods immediately following rain (e.g. Camillo *et al.* 1983; McInnes *et al.* 1986). Good seed-soil contact increases the soil's capacity to supply water to a seed at a given potential (McWilliams and Phillips 1971; Dasberg and Mendel 1971; Hadas 1977*a*; Sheldon 1974), and hence the ratio of seed to soil particle size has a significant effect on the response observed in germination under moisture stress experiments. However, seed size of *E. delegatensis* and the seed size of *E. sieberi* (tested by Bachelard 1985), which displayed apparently

greater sensitivity, are approximately equal, ruling this out as a factor. Gibson and Bachelard (1986*b*) note that *E. sieberi* has a heavily suberised inner integument that restricts water uptake. *Eucalyptus delegatensis* also has a suberised inner integument (Guaba and Pryor 1958), however water passes through this integument freely (Grose 1963). Possibly it is this greater seed coat resistance difference which makes *E. sieberi* more sensitive to falling matric potentials.

The ability of seeds to germinate in the field will be affected by soil contact and relative humidity as well as matric potentials. This study showed germination to be greatly retarded by low humidities. Where seed-soil contact is poor, the rate of drying relative to the rate of imbibition may prevent the seed from achieving or holding relative water contents sufficient for germinative processes to progress.

The interaction of temperature and water potential on germination processes has been noted in the germination response of a number of species (Kaufmann and Ross 1970; Weerakoon and Lovett 1986; Wurr and Fellows 1987). However, contrary to the finding of others, this study found that the germination capacity of samples at, or near to, the optimum temperature for germination was affected by less severe stress levels than samples germinating at either super- or sub-optimal temperatures (Fig. 2.10). One possible implication of this is that the seeds that are most sensitive to departures away from the optimum germination temperature, are also the most sensitive to water stress.

The imbibition experiments (Fig. 2.17) suggest that the impediment to germination at lower osmotic potentials is not entirely related to the seed's ability to achieve a sufficient level of hydration to commence physiological activity, but probably to growth related processes. Although seed germination was severely impeded at water potentials of -0.5 MPa, seed water uptake was not. Water uptake at more severe levels of water stress was, however, retarded, but by this stage no germination occurred. Gibson and Bachelard (1986*b*) working with *E. sieberi* also found that water uptake during the first phase of imbibition was unaffected by substrate water potentials within the range which germination rate and germination capacity were affected. These results are somewhat contrary to the main body of germination literature (e.g. Owen 1952; Hadas 1977*b*). Nevertheless, this first phase during which the water potential of the seed and the water potential of the soil solution are at their maximum difference is the least likely to be sensitive to modest water potential deficits. Gibson and Bachelard (1986*b*) found that even after seeds have completed the first phase of imbibition,

seed water potential is still approximately -1 MPa, and that the water potential of the hypocotyl and the cotyledons is approximately -5 MPa, a sufficiently strong gradient to allow water movement under at least modest levels of water stress. The inhibition of germination at levels of water stress that do not impede water up ake is probably due to the inhibition of pre-emergence growth processes which have been found to be more sensitive than germination initiation stages (Hegarty 1977; Dell'Aquila 1992).

The priming of *E. delegatensis* seeds by holding in polyethylene glycol solutions unfavourable for germination, in common with the response of seeds of many other plants (Bradford 1986; Gray *et al.* 1990), increased the germination rate. The increase in germination rate at lower matric potentials was due entirely to the shortening of the time to initiation of emergence and it was only at the highest matric potential, -0.5 MPa, that germination was more synchronous. It has been hypothesised that the increase in germination rate associated with priming is due to the progress of germination processes in phase II of germination (phase I, II & III *sensu* Bewley and Black 1982), the phase of germination following the initial rapid uptake of water. The observed uniformity of germination of seeds following priming is believed to be related to seeds completing this phase of germination and being checked before entering the next phase of germination associated with radicle protrusion, phase III (Gray *et al.* 1990). Rapid water uptake of *E. delegatensis* in the absence of stress occurs until the relative water content reaches 40%, and this seems to be a necessary threshold for germination to occur. Seeds in solutions of water potential above -1 MPa attained this level rapidly and commenced phase II of germination within 24 hours. After 28 days seeds in solutions of -1 MPa had only just attained a relative water content of 40% and seeds in solutions of -2 and -3 MPa still had not. This is probably the reason why germination of seeds primed in these solutions for 7, 14 and 28 days did not display increased synchronisation of gennination relative to non-primed seeds. The increase in rate of germination once transferred to 0 MPa in these cases is probably due predominantly to the shortening of the time to commencement of phase II as a resul of the higher initial relative water content.

Similar to the germination response of *E. sieberi* (Gibson and Bachelard 1988), *E. delegatensis* is capable of withstanding intermittent drying. However, unlike *E. sieberi* which appears capable of 'stop-go' germination, that is, making progress towards germination in each wet cycle, *E. delegatensis* appears unable to shorten time to germination by being primed during a preceding wet period. Hegarty (1978), after reviewing the literature regarding the hydration and

dehydration of seeds, concluded that although different investigators have reported favourable, neutral and unfavourable results of such treatments, generally the rate of germination is increased after treatment, although at some point in the germination process desiccation becomes damaging. It is possible that the early rupturing of the seed coat relative to germination time observed in *E. delegatensis* may have partially contributed to this study's result. When imbibition had progressed beyond about 60 hours, drying of seeds caused a significant reduction in germination capacity (Fig. 2.18). The first seed coats were observed to rupture after approximately 60 hours, and it seems likely that the reduction in germination capacity was caused by the death of these individuals. As the elapsed time since imbibition commencement increased, an increasing proportion of seeds ruptured their seed coats, and were killed in any subsequent drying episode. This would also have an effect on the perceived germination rate, since those seed already having made substantial progress to germination would be killed, and only those seeds yet to fully commence germination would survive to commence germination in the next hydration cycle. *Eucalyptus delegatensis* appears to require approximately 100 hours without drying, and 80 hours after the rel tive water content had reached 40%, for the least recalcitrant seeds to germinate. Longer is required if soil matric potentials are less than or equal to -0.1 MPa or the humidity is low. Seeds can be dehydrated within the first 60 to 80 hours of imbibition with only a relatively minor reduction in the number of viable seeds. Upon drying seeds will require another prolonged wet period before again becoming vulnerable to death as a result of dessication. It seems unlikely therefore, that light rain showers in the field will reduce the amount of viable seed available.

2.4.2 Inter- and Intra-Seedlot Variation

Germination rate responses to temperature and moisture stress were relatively consistent between seedlots from widely different geographic origins. Germination capacity responses, however, differed considerably between seedlots, most markedly in dormancy distribution within the seed population, but also in germination capacity response to moisture stress. The seedlots from the driest sites, M38 and L17, exhibited the least sensitivity to moisture stress, although it is only the response of the L17 provenance that was significantly different. A similar relationship between seed germination sensitivity to moisture stress and parent habitat was found between different different eucalypt species by Bachelard (1985) and between provenances of eucalypt species (Gibson and Bachelard 1987). It is interesting to note that the L17 provenance displayed the

least sensitivity in germination capacity to both low temperature and moisture stress. It has been observed (Mayer 1986) that the ability of seeds to germinate at low temperatures is often correlated with the ability to germinate under conditions of low water potential. The proportion of dormancy within the seedlots also appeared to be related to the environment of origin. Seed from the warmest seed zone, L17, was least dormant, while seed from the coldest seed zone, M50, was most dormant, suggesting that winter mortality may be a selective influence on the proportion of seed dormancy within the seed population. The proportion of seed dormancy also accords well with the means of those for the respective sub-regional groupings identified by Boland and Dunn (1985). In this study seed from south-east Tasmania displayed the lowest proportion of dormancy, followed by nearly equivalent dormancy in the other two identified sub-populations. The proportion of dormant seed in the populations in this study was found to be far higher than Boland and Dunn (1985) found in their study, and far more in line with the more extensive data set of the Forest Commission, Tasmania (Lockett, 1991). Current Tasmanian eucalypt seed-testing procedures stratify seed for 28 days (Lockett 1991). It was found in this work that although this would give an accurate assessment of the viability of most seedlots, it may substantially under-estimate the viability of highly dormant seedlots.

While difference in germination attributes can be associated with the geographic origin of seed, considerable variation in germination response exists within seedlots. Seedlots show continuous and unimodal variation in germination response to soil water potential, temperature, and duration of stratification. The complete ecological range of the species is subject to both unseasonable frosts and dry periods, and hence the variation between seasons at a site is likely to be as great as the variation in average conditions between sites. This seasonal variation is magnified at the microsite level, where seeds germinating on small mounds or hillocks, for example, may experience far drier conditions during germination than seeds in hollows. It is possible that the combination of seasonal variation and regeneration microsite heterogeneity maintains the high degree of variation in germination response within seed populations. While it is undoubtedly true that such variation protects against incidental fluctuations in germination conditions and represents a form of insurance in the face of temporal environmental variability (Harper 1977; Westoby 1981; Venable 1985), it seems possible that selection at the microsite level also acts to maintain the diversity of germination response within the population. Investigation of morphological traits in mature *E. delegatensis* has indicated that genetic diversity

within the species appears to be primarily within, rather than between populations (Moran and Hopper 1987). The geographic scale at which environmental factors such as soil moisture act to select for some germination characteristics is likely to be quite different to that acting on morphological characters because of the profound impact of microsite variation on the seed germination environment and the comparatively rapid response of seed germination to transient weather events. The questions of the partitioning of genetic variance into between provenance and between-tree components and the importance of microsites in maintaining the diversity of germination response are investigated in subsequent chapters.

This species displays a number of germination characteristics which minimize the chances of germination at times when the probability of establishment is low. Seedlots which come from cold areas have a high proportion of dormant seed which requires a long period of cool moist stratification to germinate. Natural seedfall is in late summer or early autumn (Grose 1957*a*) and a proportion of dormant seed ensures that germination is spread between autumn and spring, preventing the total genetic stock being removed by one untimely frost event. The requirement for continuous imbibition for at least three days before seeds suffer excessively from dehydration, and the ability of the seed to survive a number of short cycles of wetting and drying prevents short rainfall events in summer or autumn from destroying ground-stored seed. These traits and the ability of seed to germinate and survive over a range of temperature and soil moisture conditions undoubtedly accounts in part for the geographic success of the species.

Chapter 3: Variation in seed germination characteristics: inter and intra site components

3.1 Introduction

Isoenzyme and morphometric work on *Eucalyptus* has indicated that for widespread species, such as *E. delegatensis*, genetic diversity primarily resides within rather than between populations (Moran and Hopper 1987). However, marked genetic and morphological differentiation has been shown to occur between Tasmania and mainland Australian populations of *E. delegatensis* (Boland *et al.* 1982; Moran and Bell 1983; Ohmart *et al.* 1984; Boland and Dunn 1985) to the extent that these have been variously proposed as different species (Hooker 1847, 1856) and sub-species (Boland 1985) at different times. Consistent with the general finding for widespread eucalypts, however, populations are believed to be genetically similar within each of these areas (Moran and Bell 1983). Nevertheless, it was shown in Chapter 2 that substantial variation in germination response to environmental conditions occurred between Tasmanian provenances of *E. delegatensis*, and that the response of seedlots to temperature and water potential could be related to their geographic origins. Similar relationships have been demonstrated for *Eucalyptus* by a number of workers (e.g. Ladiges 1974; Bachelard 1985; Gibson and Bachelard 1987). Substantial within provenance variability was also found in the responses to environmental conditions (reported in Chapter 2). Because the seedlots used were bulked collections from a number of trees it was not possible to identify to what extent this was the result of between-tree variability, within-tree variability or experimental noise. The partitioning of variation in trees into within-tree and between-tree components has been investigated for morphological characters such as leaf and seed capsule size (e.g. Potts and Reid 1985; Potts 1989), seed physical characteristics (e.g. Briand *et al.* 1992) and adult plant physiological response (Tibbits and Reid 1987). The partitioning of variation of seed physiological response to environmental conditions has received less attention.

A relationship between a site's environment and the genetic composition of the resident plants on a very local level has been demonstrated in a number of studies. For example allozyme frequency, seed weight and time to seed set have been shown to be correlated with microsite moisture (e.g. Linhart 1974; Solbrig and

Simpson 1974; Hederick *et al.* 1976; Hamrick and Holden 1979; Nevo *et al.* 1981; Nevo *et al.* 1988), life span and reproductive strategy related to site disturbance regime (Law *et al.* 1977), and life span and plant morphology related to site nutrition (Snaydon and Davies 1972). Studies in which the variation of a range of morphological (e.g. Potts 1989) and life-history (e.g. Imam and Allard 1965) characteristics of a species have been studied have shown that the partitioning of the variability into within and between populations can vary widely between characters. The geographical scale at which selective pressures are operating might provide some explanation for these differences. Characters that might be selected against in a predictable and uniform environment may be retained within a population through the action of heterogeneity that is unpredictable in time and space (Hartgerink and Bazzaz 1984). Hence, characters affected by local or micro-scale spatial heterogeneity might be expected to display a higher proportion of within-provenance variability than characters that are affected by conditions that are uniform at the stand or provenance scale.

In this chapter the partitioning of variation of a number of seed germination traits into within and between site components is considered for seed samples from six trees at each of two disparate sites. Besides obvious implications for the way in which seed is collected for sowing in regeneration operations, an understanding of the partitioning of variability of seed germination characteristics has clear implications for seed germination modelling. It is apparent from Chapter 2 that a separate modelling solution could potentially be required for each provenance. If substantial between tree, within provenance, variability exists then unless an average provenance, or seedlot response is all that is required (as will be the case for most management questions) individual tree variability will need to be taken into account. Instances where this will be necessary are principally theoretical questions such as the long term implications of sowing off-site seed or questions regarding the evolution of dormancy responses.

3.2 Methods

Seed was collected from six trees at each of two sites. The first site was located near Ben Nevis on the Camden plateau in the north-east highlands of Tasmania (FORESTIER 1:100 000 Mapsheet 525 205) and the second in the Eastern Tiers, inland of Bicheno (BREAK O' DAY 1:100 000 Mapsheet 990 610) (Fig. 3.1). Previous work investigating frost tolerance had indicated that the response of adult trees from these locations was substantially different (Webb *et al.* 1983; Hallam and Reid 1988). Macroclimatic data for both of these sites from the

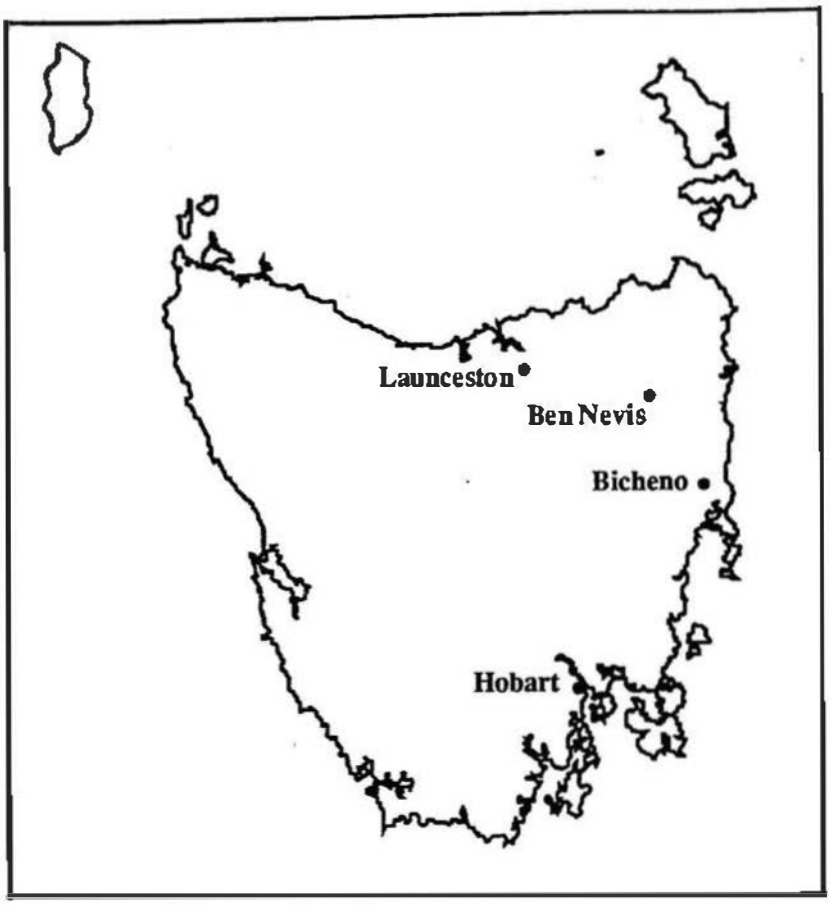


Fig. 3.1. Location of sites from which seed was collected for experiments in Chapter 3.

process based climate model BIOCLIM (Busby 1991) are given in Table 3.1. Adult trees typical in form and vigour of those in each provenance were selected. Trees were at least 50 metres apart. Capsules of the one seed crop were collected from a range of branches on each tree, and were subsequently air dried to extract the seed.

Because each seed of *E. delegatensis* weighed so little (≈1 mg per seed) seed weight was measured as the sum of the weights of 10 randomly selected seeds. Following weighing, each seed particle in the sample was squash tested to check that it was indeed a viable seed. If a seed did not appear viable a new sample of 10 seeds was selected.

To examine dormancy, seeds were sown after being stratified for 0, 14, 28 and 56 days. To examine the response to water stress, non-stratified seeds were sown onto filter papers saturated in polyethylene glycol 6000 MW solutions of 0, -0.25, -0.5 and -0.75 MPa. These solutions were topped up daily and filter papers and solutions changed every week. Germination of non-stratified seed was recorded

daily to provide a measure of germination rate as well as germination capacity. Germination of other treatment combinations was recorded at least once a week. All germination tests were conducted on filter papers inside petri dishes maintained at a temperature between 15° and 22.5°C, with a 14 hour photoperiod of approximately 200 μmol m^{-2} sec^{-1} supplied by a mixed incandescent and fluorescent light source. Four weighed replicates of seeds were used for each treatment combination. Preliminary squash tests had indicated that the viability of the seed from the Ben Nevis site was significantly lower than from the Bicheno site. Within the constraints of total collected seed weight, 1.0 g of seed was used for each Ben Nevis replicate and 0.5 g of seed for each Bicheno replicate. Low seed volumes in some cases resulted in less seed being used per replicate. The viability of the seed from one of the Ben Nevis trees (tree 4) was very low and it was not used in subsequent analyses.

Germination capacity was defined as the total number of seeds to have germinated when seven days at the prevailing conditions failed to give rise to additional germination. For analysis this was converted to a percentage of the highest germination capacity observed under any set of test conditions or in the case of the dormancy experiments the percentage of seeds to germinate relative to the 56 day stratification treatment. Consequently germination capacity as expressed in this chapter is a relative measure. Germination rate was measured as the time taken for 50% of the final total emergents to germinate, *t50*. Results were analysed using a mixed model with the RANDOM option for the GLM module of the statistical package SAS (SAS 1989). A nested design with trees nested within provenances was used. Stratification and water potential were treated as fixed effects and provenance and tree as random effects. Petri dishes constituted the experimental unit. The degrees of freedom associated with the appropriate *F* value were computed using Sarrerthwaites approximation. Variance components

Table 3.1. Macroclimatic data from BIOCLIM (Busby 1991) for the experimental sites.

Climatic Variable	Ben Nevis	Bicheno
Annual mean temperature (°C)	7.3	10.4
Maximum temperature of warmest month (°C)	18.1	20.7
Minimum temperature of coolest month (°C)	-0.85	2.3
Mean annual precipitation (mm)	1542	992
Precipitation of wettest month (mm)	200	95
Precipitation of driest month (mm)	58	60
Wettest quarter precipitation (mm)	547	270
Driest quarter precipitation (mm)	212	213

were estimated using the VARCOMP procedure of SAS, using the method=REML option. Spearman's correlation coefficient was used to identify the correlation of characters. The dormancy and water stress response of germination were converted to a single figure index. The proportion of viable seed that germinated without stratification was used to characterize the dormancy profile of seeds, and the proportion of seeds that germinated at -0.25 MPa was used to assess tolerance to declining water potential.

3.3 Results

3.3.1 Seed Weight and Germination Rate

The germination rate of all trees tested was similar (Fig. 3.2, Table 3.2). Considerably more variability existed in the mean response of trees from the Ben Nevis site than trees from the Bicheno site. This may be partially a result of the lower numbers of fertile seeds per replicate. The variation in germination rate due to site was negligible, and approximately 23% was attributable to trees within sites. Within any one replicate there was a considerable spread of germination times. Seed germination began 7 days after the commencement of imbibition and was generally not completed until 28 days later. The maximum variation in germination rate between trees and provenances was only a few days. While some of the within-test sample variation may be due to small variations in conditions in the petri dish, it seems clear that substantially more variation in germination rate exists within the seed from one tree than there does in the seed from different trees within a site, or between the sites tested.

Seed weight varied significantly between and within sites (Fig 3.3, Table 3.2). Seeds from the Bicheno site were significantly larger than seeds from the Ben Nevis site, and displayed greater between tree variation in seed size. Separate samples of 10 seeds taken from the same tree displayed little variation in weight, and this is indicated by the very low error variance component (Table 3.2). This may be partly a result of the averaging effect inherent in taking replicates of ten seeds, but seems also to imply homogeneity in seed size within the one seed crop from the one tree.

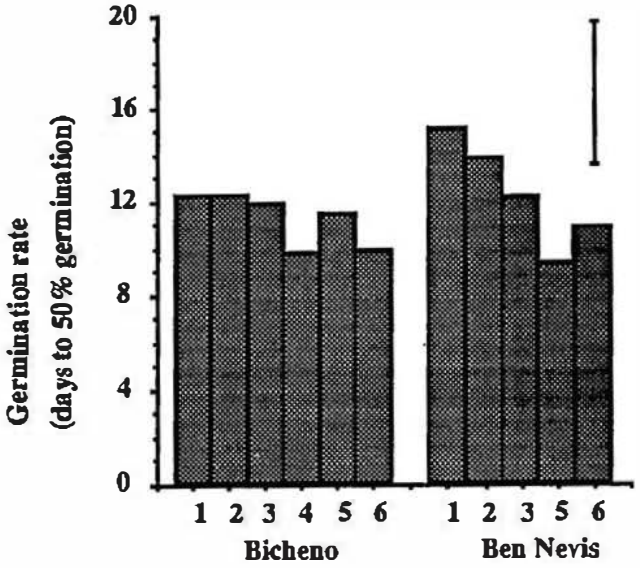


Fig. 3.2. Within and between provenance variation in germination rate. The error bar is the Tukey-Kramer least significant difference for multiple comparison.

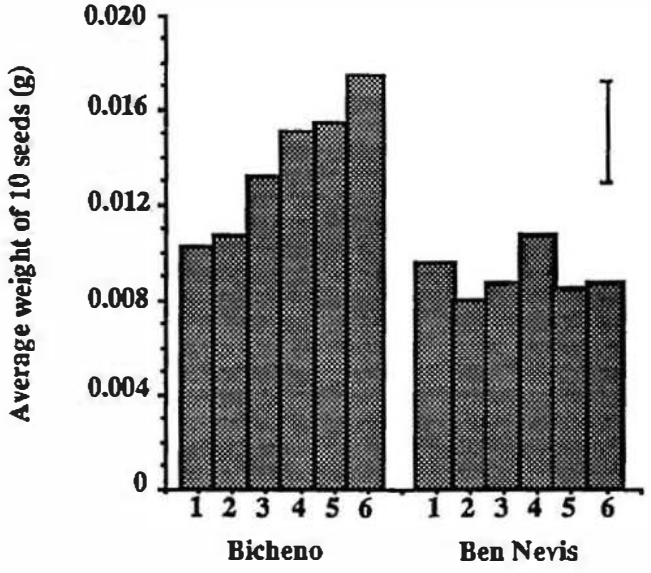


Fig. 3.3. Within and between provenance variation in seed weight. The error bar is the Tukey-Kramer least significant difference for multiple comparison.

3.3.2 Dormancy

Seed from both sites, and trees within sites, responded significantly differently to stratification (Fig. 3.4, Table 3.2.) Seed from the Ben Nevis site had a substantially higher proportion of dormant seed. Without prior stratification only 30% of the viable seed from the Ben Nevis site germinated, compared to 80% of the seed from the Bicheno site. The tree from the Bicheno site that yielded the seed with the highest proportion of dormancy, tree 2 with 45% of seed dormant, had a lower proportion of dormant seed than did the least dormant seed sample from the Ben Nevis site, with 52% of seed dormant. The seed from one tree at the Bicheno site (tree 3) was totally without dormancy and the seed from another tree (tree 2) was rendered non-dormant following stratification for 14 days. The seed from only two of the six trees from the Bicheno site (trees 6 and 1) required more than 28 days to give complete, or very near complete, germination. By contrast the seed from only two of the six trees collected from the Ben Nevis site were unimproved in germination capacity by stratifying for more than 28 days.

Along with the different proportions of innate dormancy, there was a significantly different pattern of response to stratification both within ($P<0.01$) and between sites ($P<0.001$: Table 3.2). Variation in the rate at which dormancy was relieved was overwhelmingly concentrated at the between site level, with the stratification of trees within sites, particularly seed from trees at the Bicheno site, being comparatively homogeneous. A greater variability in the response of seed from trees from the Ben Nevis site to stratification existed. This is most evident in the difference in germination capacity following 14 days stratification. Nevertheless, although variability exists within sites in the proportion of seed that is dormant, generally each site has a similar pattern of dormancy release in response to stratification.

The marked difference in the dormancy attributes of the sites is reflected in the positive correlation between seed weight and the proportion of seed that will germinate without stratification (Table 3.3a). However, if the site from which the more dormant seed is derived is considered alone the correlation is reversed (due to the presence of two discrete clusters related to the differences in the size of seeds at each site), and at the Ben Nevis site in particular where the proportion of dormant seed was high, trees that produce smaller seed also produce less dormant seed (Table 3.3b). Clearly examination of seed from trees at more sites is required if any meaningful inference is to be drawn.

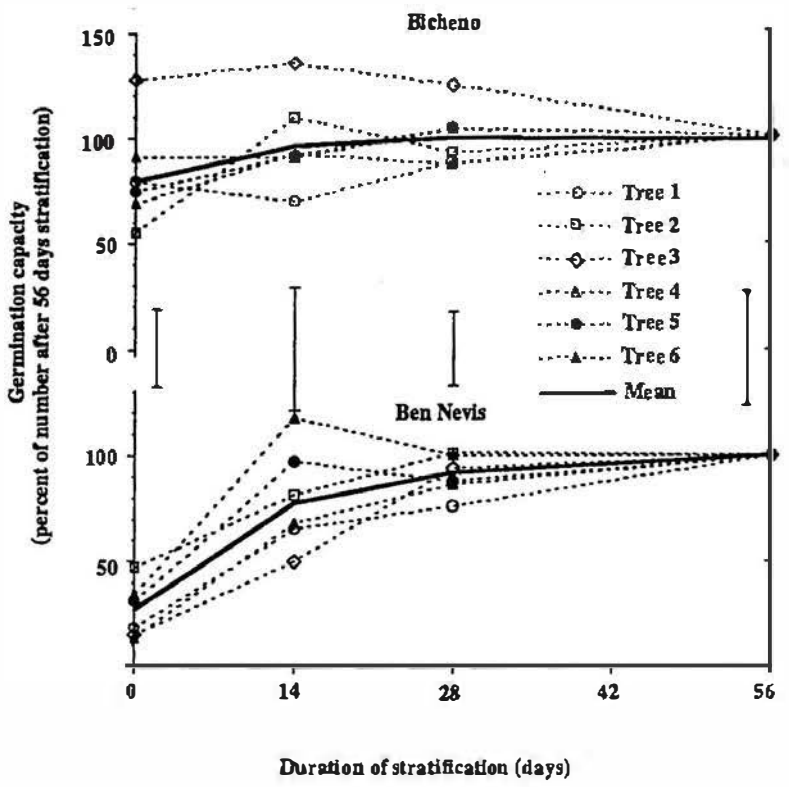


Fig. 3.4. Variation between and within sites in the depth of seed dormancy. The error bars are the Tukey-Kramer least significant difference for pairwise comparison of germination capacity across trees and sites.

Table 3.3. Correlation of seed and germination characteristics using Spearman's correlation coefficient.
Rate is the days for 50% of seeds to germinate, dormancy is the percentage of viable seeds that germinate without stratification and water stress is the proportion of seeds that germinate at -0.25 MPa Levels of significance * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

a. BOTH SITES

	RATE	*WEIGHT*	*DORMANCY*	*WATER STRESS*
RATE	1.000			
WEIGHT	-0.264	1.000		
DORMANCY	-0.282	0.655 *	1.000	
WATER STRESS	-0.409	0.627 *	0.909 ***	1.000

b. BICHENO

	RATE	*WEIGHT*	*DORMANCY*	*WATER STRESS*
RATE	1.000			
WEIGHT	-0.657	1.000		
DORMANCY	-0.200	-0.143	1.000	
WATER STRESS	-0.371	-0.314	0.600	1.000

c. BEN NEVIS

	RATE	*WEIGHT*	*DORMANCY*	*WATER STRESS*
RATE	1.000			
WEIGHT	0.400	1.000		
DORMANCY	-0.100	-0.800 *	1.000	
WATER STRESS	-0.500	-0.800 *	0.700	1.000

3.3.3 Response to water potential

Seeds from different sites showed markedly different germination sensitivities to the water potentials tested (Fig 3.5, Table 3.2). A reduction in soil water potential from 0 to -0.25 MPa decreased the germination capacity of the seed from all trees from the Ben Nevis site to below 25%. The germination capacity of all trees from the Bicheno site exceeded 40% at this water potential, and more than 25% of the seed from four of the trees germinated at a matric potential of -0.5 MPa.

The variability in the mean germination capacity across the range of water potentials tested (indicated by the site by environment and tree within site by environment variance components of germination capacity) resided overwhelmingly between sites with the mean response of trees within sites being similar (Table 3.2). Similarly the rate of decline of germination capacity with increasing moisture stress (the interaction between site and level of stress, and tree within site and stress level) again

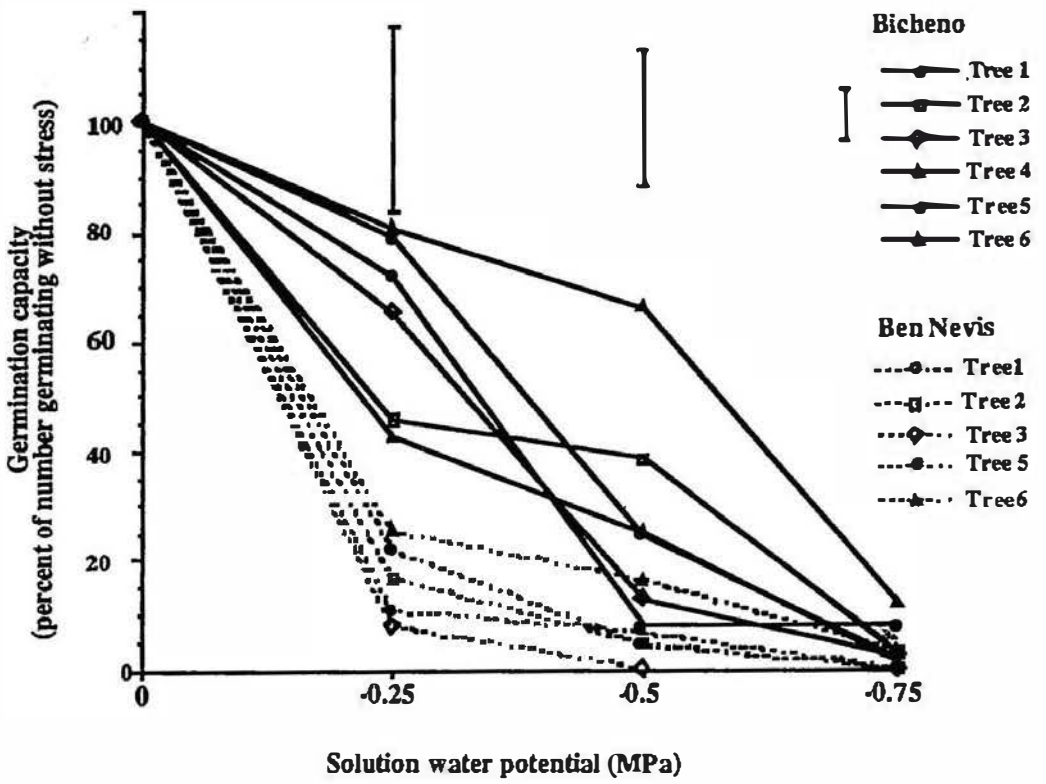


Fig. 3.5. Variation between and within sites in the ability of seeds to germinate at various solution osmotically induced water potentials. Error bars are the Tuley-Kramer least significant difference for multiple comparison.

indicates that variability in response to the levels tested was predominantly at the site level. However, if each site is considered individually it can be seen that while all seed collected from trees at the Ben Nevis site responded similarly, the pattern of response of seed from different trees at the Bicheno site was significantly different ($P \leq 0.0001$).

There was a strong correlation at both sites between seed dormancy and the ability of seed to germinate whilst subject to water stress (Table 3.3b&c). Seedlots with a higher proportion of dormancy, were more susceptible to reduced water potentials.

3.4 Discussion

In this study, trees from the wetter site (Ben Nevis) produced significantly smaller seed than did trees from a drier site (Bicheno). While seed from the drier site was larger, this was not reflected in the within-site variation in germination response

to water stress. At Ben Nevis, trees that produced large seeds also produced seeds that were less tolerant of osmotically-imposed water stress. The proportion of variation in seed size due to within-site effects (28%), whilst significant, was small compared to the proportion due to the site effect (66%). Seed size has been shown in some studies to vary with site drought risk (Baker 1972; Ladiges 1974). It has been suggested that seedlings of species that develop from large seeds will establish more successfully under dry conditions than will those from small seeds (Grose and Zimmer 1958; Baker 1972) This is possibly because a larger seed enables a seedling to produce more rapidly an extensive root system and hence more effectively utilise soil moisture (Silvertown 1982). These relationships, however, do not always apply. Ladiges (1974), for example, found that seed from trees of *E. viminalis* growing in a drought-prone environment was smaller than seed from trees growing under more mesic conditions. Further, reinterpretation of Baker's (1972) results (Westoby *et al.* 1992) indicated that the relationship between seed size and habitat in that study may be related coincidentally to habitat by way of lifeform rather than directly as a response to the moistness of the site. Other factors, such as the ratio of seed to soil particle size (Sheldon 1974), influence the ability of a seed to take up and retain moisture. Furthermore environmental influences during seed set, such as decreased water availability to mother plants (e.g. Mekel *et al.* 1984; Sawhney and Naylor 1982), can affect seed size. Replicates of ten seeds from each tree were all similar in weight, suggesting that for *E. delegatensis,* seed weight varies largely among trees within sites rather than within trees. Similar results have been found in some other studies (Howe and Ritcher 1982; Kang *et al.* 1992), however, usually the converse situation has been found (e.g. Schaal 1980; Thompson 1984; McGinley *et al.* 1987, Michaels *et al.* 1988).

There was no significant difference between the germination rate of seeds from different trees within or between sites. Variation in germination rate was almost entirely a function of between tree variability, and this largely as a result of variation at the Ben Nevis site rather than the Bicheno site. Low numbers of germinating seeds (approximately ten seeds per replicate for two of the trees tested; trees 5 & 6), as a result of the high proportion of dormant seed, may be the cause of this variability. The variability in the time it takes individual seeds from the one tree to germinate is large. There is a difference of between three and four weeks in the time for the first and the last seed in a sample to germinate under optimum test conditions. In environments where fast growth can be achieved, early germination has been shown to result in early dominance (Black 1958; Abul-Fatih and Bazzaz 1979; Cook 1980; Campbell and Bray 1987). It may be,

however, that in a temporally variable climate, and one in which mortality hazards are distributed in many months of the year, that a spread of germination times increases the probability of some seedlings germinating at an appropriate time (Westoby 1981), and this has reduced the selective pressure for rapid emergence.

Variation in stratification response in this study was found to be predominantly between sites. This substantial site variation is not surprising given that the sites selected were contrasting in adult tree frost tolerance. It is to be expected that the most appropriate dormancy response would be determined by the temporal distribution of, and temporal variability in, seedling mortality factors (e.g. Silvertown 1982; Venable 1989). Such factors as frost frequency, are relatively consistent within sites, and it is therefore to be expected that a particular pattern of dormancy should prevail within a site. The Bicheno site, at 300 m, is mild with far fewer and less severe frosts than the Ben Nevis site, at 1100 m, and it will be shown later (Chapter 6) that autumn germinants at the Bicheno site have a greater chance of survival over winter than do autumn germinants in a frosty area analagous to the Ben Nevis site. A similar correlation between the coldness of sites and the proportion of dormant seed was shown in Chapter 2. Nevertheless, a significant amount of variability in dormancy pattern exists within sites. The primary control of seed dormancy and germination has been interpreted as acting through the maternal tissues surrounding the embryo (Mayer and Poljakoff-Mayber 1975), and common garden experiments suggest that much of the variation in germination requirements among populations may be environmentally induced (Roach and Wulff 1987). However, Karssen *et al.* (1983) have shown that it is the embryo controlled pool of abscissic acid in *Arabidopsis thaliana* that contols dormancy. It is possible, therefore, that the control of dormancy in *E. delegatenis* may have both maternal and embryo components. Greater variability in conditions that pertain to the fitness of different emergence responses in this experiment lie between sites. Consequently, while some variability due to microenvironments will occur within sites, it is to be expected that variation in dormancy due should occur predominantly between sites.

The total variation in germination response between trees at the Ben Nevis site to the range in water stress tested was slight Trees at the Bicheno site, however, varied substantially. Similar variability between trees at the Ben Nevis site may be present in the untested 0 to -0.25 MPa water potential range. The climate at the Bicheno site is drier, and seasonal changes in soil moisture greater than at Ben

Nevis. At the Ben Nevis site, soil stays moist for most of the year. This site difference may have lead to the highly significantly different pattern of response of seed germination to osmotically-induced water stress. The substantial variability between trees from the Bicheno site may indicate the maintenance of response variability as a result of seedbed spatial heterogeneity. Within and between sites, the ability to germinate under water stress was negatively correlated with germination rate, albeit only loosely. All things being equal, it is to be expected that early emergents will have a competitive advantage in a homogeneous environment and consequently seed that germinated late would be selected against. The heterogeneity in soil moisture conditions caused by microsite, and possibly by temporal variability, may, however, maintain response variability in the population. Similar observations have been made about seed size and microscale patch effects (Burdon 1980; Solbrig 1981; Hartgerink and Bazzaz 1984). At the Ben Nevis site where soil moisture conditions are more predictable, response variability over the range tested is small. Nevertheless, variability in response to lesser levels of water stress may still exist in the population.

The characteristics examined in this study varied in the proportion of variability due to site and within site effects at the response levels tested. Sampling at the site level was limited and only two sites, both occurring within the same regional provenance grouping of Boland and Dunn (1985), were examined. They represent, however, substantially different environments (Table 3.1). In provenance trials the frost tolerance of seedlings from seed collected from these sites have been shown to differ substantially in frost tolerance (Webb *et al.* 1983; Boland and Dunn 1985; Hallam and Reid 1988). It must also be noted that maternal parents may influence seed characteristics through both genetic and maternal environmental effects (Schaal 1984; Roach and Wulff 1987; Wulff and Bazzaz 1992; Mazer and Wolfe 1992). It is not possible in the present study to separate the influence of maternal environment effects on seed characteristics from genetic influences. The findings of this work nevertheless indicate the significance of the some of the sources of variation in germination characteristics and provide a basis for future work.

In this study, the proportion of variation in seed and germination characteristics attributable to between and within site effects differed with the characteristic examined. This, to some extent, could be related to the scale at which selective forces were presumed to operate. The proportion of dormant seed produced by trees and its response to stratification, for example, varied predominantly between

sites, the scale at which the fitness of particular dormancy release response would be expected to vary. The variation in response to water stress, by contrast, appeared to vary both within and between sites. This is consistent with the scales at which soil mositure varies: as well as variation in rainfall patterns at the scale of kilometres or more, soil moisture varies locally as a result of microtopographical variation. Nevertheless, a substantial amount of variation in response existed within the seed collected from the one tree. Interestingly, whilst this has been noted for seed size in other studies (e.g. Michaels *et al.* 1988), in the present work, seed size was the most consistent of characteristics within trees. The extent to which this within tree variability of seed germination characteristics is an evolutionary response to spatial and temporal heterogeneity in the regeneration niche or developmental noise is unclear. Seed characteristics have been shown to vary between years (Grose 1963), with canopy position (Mazer *et al.* 1986), with position in the ovary (Schaal 1980), and with environmental conditions (Roach and Wulff 1987). It has also been suggested, however, that the time variation of some physiological responses, such as the initiation of cancer in cells (Rashevsky 1960) and the induction of flowering in peas (Reid and Murfet 1980), is determined by accidental or random fluctuations that affect threshold responses in essentially identical biological entities. Indeed, this later phenomenon has been used by some workers to model the spread of germination times within seed populations (Shibuya and Hayashi 1984) and the germination of bacterial spore populations (Leblanc and Lefebvre 1984). The extent to which these alternatives are related is unclear and the question of whether processes are truly stochastic, or are deterministic, but susceptible to very minor perturbations during development, requires further investigation. Nevertheless, the net result of these influences during maturation, whether stochastic or deterministic, is that seed germination characteristics such as seed dormancy, if is strongly determined by maternal tissue effects, may display very low heritability (e.g. Arthur *et al.* 1973).

Chapter 4: The effect of microsite variation on seed germination and seedling survival.

4.1 Introduction

The forest floor is highly heterogenous in its physical and chemical environment (Arp and Krause 1984; Lechowicz & Bell 1991). The chances of a seed germinating will be dependant, at least in part, upon the characteristics of the microsite it occupies. However, the suitability of microsites may vary with climatic conditions and hence the time of year. The success of germination and seedling survival may therefore be determined by the interaction of microsite and climatic factors (e.g. Potts 1986). In this sense a 'safe site' for germination (Harper *et al.* 1965) is determined in both space and time. An assessment of the significance of the importance of spatial heterogeneity on germination rate and success is integral to the prediction of field emergence.

Microsites can affect seedling distribution by influencing seed survival, seed germination and subsequent seedling survival. Protected microsites will be buffered from fluctuating soil conditions which may adversely affect seed survival before a regeneration opportunity. Seeds may partially complete germination in protected microsites during adverse conditions and may be able to germinate more rapidly when favourable conditions arise. This may aid establishment before the next cycle of unfavourable conditions. Microsites that have favoured seed germination will not, however, always be suitable for subsequent seedling establishment (e.g. Ashton and Willis 1982; Read and Hill 1988; Barker 1992) and the nature of the microsite may change with time so that survival is later threatened (e.g. Potts 1986). The dependence of seedlings on protected niches to survive climatic adversity, however, might be expected to decline with time as the plant develops the ability to exploit environments beyond the immediate germination environment.

Regeneration of eucalypts has been observed to be influenced by microsite characteristics, although differences have not been quantified. Field germination of eucalypts in Tasmania and montane parts of south-eastern Australia is principally confined to spring and autumn when the soil is both warm and moist (Lockett 1991). The small amount of germination that occurs in summer and

early autumn is confined to sheltered positions (Cunningham 1960). Germination at any time of the year, but most particularly in summer, has been found to be favoured by shading from logging residues (Jacobs 1955; Cunningham 1960; McCormick 1990) and the importance of microhabitat is influenced by site factors such as aspect and topographical position (Potts 1986). Regeneration is impeded on areas subject to profile inversion, puddling or compaction during logging (Cremer 1962; Calais & Kirkpatrick 1983; Williamson 1990). Seeds germinating on substrates that offer resistance to root penetration may die from desiccation before roots penetrate the soil (Sheldon 1974) and seedlings growing in heavy soils have greatly reduced root systems and are highly susceptible to drought (Williamson 1990). The humidity of microsites, mediated by season and topography, therefore, appears to be of critical importance to eucalypt seedling establishment.

In this study the spatial pattern of germination of *Eucalyptus delegatensis* in the field was examined following sowings made in different seasons. Microsites were created on artificial seedbeds in the glasshouse to test the importance of microsite on germination and seed survival under differing regimes of watering. The interaction of seedling age and microsite on survival during prolonged drought was subsequently tested. Finally the extent to which selection at the microsite level influences the characteristics of seedlings was investigated.

4.2 Methods

4.2.1 Field Study

Seed was sown on 1 m^2 plots within an area of clearfelled forest near Bicheno, Tasmania (Universal Grid Reference 55GEP932645) on four separate dates: 22/3/1989 (autumn), 21/6/1989 (winter), 3/10/1989 (spring) and 6/2/1990 (summer). Prior to each sowing the plot was disturbed by hand hoeing so that all existing vegetation was removed and all large clods were broken. Details of the Bicheno site are given in Chapter 6. At each sowing time, three plots were each broadcast sown with 6.7 g of seed (900±20 viable seeds). Details of the germination characteristics of the seedlot sown (seedlot E00080 from M36 seed zone) were given in Chapter 2. Twelve months after the last sowing date, the number of seedlings surviving on different microsites within each plot was determined. This was done by dividing each plot into 100 cm^2 units, allocating the unit into one of the three classes, hillock, depression or flat, and counting the number of seedlings within that unit. Because the plots had been previously

hoed, the microsite units were distributed more or less randomly across the plot and occurred with approximately equal frequency.

4.2.2 Germination and establishment on artificial seedbeds

Four artificial seedbeds were created in trays 1m x 1 m x 15 cm. Each tray was filled with potting mix (50% sandy loam, 40% peat moss, 10% coarse sand, with less than 1% by weight Osmocote fertilizer, dolomite and blood and bone) over a 2 cm layer of gravel and sand. The bottom of each tray was pierced with approximately 25 drainage holes, and each tray was tilted slightly to facilitate drainage. Six replicates of six microsites were laid out as a Latin square in each tray (Fig. 4.1. Plate 4.1.). The 100 cm^2 microsites created were designed to simulate seedbed types that occur naturally following logging and artificial seedbed preparation. The first three microsite types were 'hillock', 'depression' and 'flat'. Hillocks were created by artificially raising the soil surface 2 cm and supporting with a perimeter of cloth tape. Depressions were created by excavating to a similar depth and once again supporting with cloth tape. The 'flat' microsites, effectively the control, were unchanged areas of the tray. A fourth microsite, 'clay', was created by removing a 100 cm^2 core and replacing with potting mix to which had been added 30% by volume kaolin. This treatment simulates soil profile inversion due to severe disturbance. A fifth microsite, 'shade', was created by the placement at 2 cm intervals of east-west running 5 cm high baffles made from thin aluminium orientated at 20 degrees from the vertical. This treatment simulates shading of the seedbed by residual slash following logging. The final microsite, 'shallow', was created by placing an aluminium tile 1.5 cm below the soil surface, effectively restricting the profile depth. Each microsite was surrounded by a 5 cm buffer.

On each microsite, 0.15 g of seed (approximately 20 viable seeds) of the same seedlot as used in the field experiment was sown. Two of the trays were watered twice daily with a micro-fine jet, and two of the trays were watered intermittently, such that the surface of the soil on flat microsites was dry to touch at the end of every drought episode. The seed of *E. delegatenis* is small in relation to the surface roughness of the potting mix seedbeds, with the exception of the kaolin enriched microsites, and seeds were observed to settle quickly into the surface soil matrix. Droplet sizes used in the watering were too small to move seed particles by saltation, and watering rates were kept sufficiently low to avoid seed wash. No germination was observed in buffers, suggesting seed movement was very slight. Six weeks after the commencement of the experiment, after no further germination had been observed for a week, the two trays previously intermittently

watered were watered twice daily for three weeks. By this time no seeds had been observed to germinate for five days and germination was considered to be complete. One of these trays was then transferred to a cold room and chilled for eight weeks at 5°C and then transferred back to the glasshouse to see if the seeds which remained ungerminated were dormant and would germinate following stratification.

The trays that had been continuously watered were thinned to one seedling per microsite, and a further two sowings were made at monthly intervals and one seedling from each sowing retained per microsite. Each microsite was left with three seedlings, one with the cotyledons just emerging, one with fully expanded cotyledons and the second leaf pair initiated and one with two fully expanded leaf pairs (Plate 4.2). Watering was ceased and the survival of seedlings was recorded on a daily basis. Seedlings were considered to be dead when all above ground tissue was dehydrated.

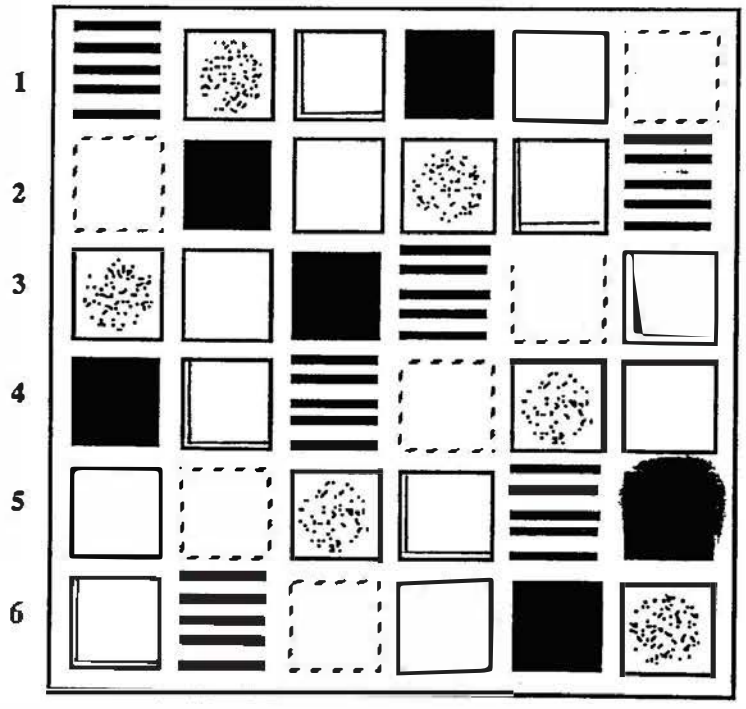


Fig. 4.1. Layout of each Latin square in the artificial microsites experiment. 1='shade', 2='depression', 3='clay', 4='shallow', 5='flat' & 6='hillock'.

Plate 4.1. View of artificial seedbeds showing different microsite types.

Plate 4.2. Seedling types retained on each microsite. Red marker indicates the oldest seedling with two leaf pairs; blue marker indicates seedlings with fully expanded cotyledons and the second leaf pair initiated; and the yellow marker indicates recently germinated seeds with cotyledons still expanding.

4.2.3 Microsite selection and seedling characteristics

Seeds were allowed to germinate on filter papers in petri dishes irrigated with polyethylene glycol 6000MW solutions with water potentials of -0.25 and -0.5 MPa. Seeds that germinated in each solution were discarded. Remaining seeds from the -0.5 MPa solution were then transferred to a solution of -0.25 MPa, and seeds from the -0.25 MPa solution were transferred to a 0 MPa solution, and a new batch of seeds was introduced to a -0.5 MPa solution. Subsequent germination thus gave rise to three sets of seedlings: seedlings that arose from seeds that could only germinate at solution potentials of greater than -0.25 MPa, those from seeds that could germinate at potentials below -0.25 MPa but not at potentials below -0.5MPa and those from seeds that could germinate at potentials below -0.5 MPa. In subsequent discussion these are referred to as '0 MPa', '-0.25 MPa' and '-0.5 MPa' seedlings respectively. These seedlings were later arranged two to a pot. '0 MPa' seedlings were matched with '0 MPa', '-0.25 MPa' and '-0.5 MPa' seedlings, each combination being replicated three times. Seedling pairs were matched as closely as possible in leaf area and size. Following watering for a period of four weeks, to overcome any stress caused by transplanting, watering ceased. Seedlings at this time were approximately 10 cm tall. Leaf diffusive resistance of each seedling was measured immediately following the last watering, and subsequently daily. Pots dried at different rates, not always in a way related to seedling size. To simplify analysis, only three of the measurement times were selected for each pot: leaf diffusive resistance when soils were saturated, when soils were moderately dry, and finally when soils had become very dry and plants flaccid. Diffusive resistance readings were taken on the most recently, or youngest, fully expanded leaf on the sunward side of the plant using a porometer (Delta-T Devices Automatic Porometer Mk3).

4.2.4 Data analysis

The field experiment data were analysed as a split plot design using the GLM module of SAS/STAT (SAS 1989). The seasons of sowing were allocated randomly between plots and were analysed using a between-plots error term and the microsite and interaction terms were analysed using the sub-plot residual variance as the denominator of the F-test. Because residual analysis showed the data to be heteroscedastic, a logarithmic transformation was used to stabilise the variance.

The glasshouse germination results were analysed as a split plot Latin-square design using the ANOVA module of SAS/STAT (SAS 1989). Watering treatments were analysed at the tray or plot level. Row, column and microsite effects were

analysed using a within-plot error term and the interaction of watering regime and microsite was analysed using the sub-plot residual variance as the denominator of the F-test.

Seedling survival was analysed by calculating the survival distribution function, S(t), using the LIFETEST procedure of SAS/STAT (SAS 1989). This was then used to calculate the hazard function which is the derivative, f(t), of the cumulative distribution function, 1-S(t), or the probability that a lifetime does not exceed t. The homogeneity of survival curves was tested using both a log rank test and a Wilcoxon test. These tests largely avoid problems of the form of the survival function (Cox and Oates 1984). In this analysis, both microsite and age of seedlings were defined as experimental strata and the design features of row, column and tray were included as covariates.

Leaf diffusive resistances of seedlings were analysed as a repeated measures incomplete block design. The dependent variable was the logarithm of diffusive resistance. Logarithms were taken of these data to minimize the correlation between the mean and the variance. Pots were treated as blocks and the seed type origin of the seedling was used as an explanatory variable. Analysis was conducted using the REPEATED option of the GLM module of SAS/STAT (SAS 1989).

4.3 Results

4.3.1 Field Experiment

Large differences in seedling numbers were observed on different microsites in the field and an interaction with season of sowing was detected (Fig. 4.2, Table 4.1). Seedling numbers were highest in depressions for all sowing times. This difference is marked for all seasons except spring. Hillock microsites were inferior to the other microsite categories for all seasons (sowing times) except winter when they were superior to flat microsites. Microsite differences were most pronounced for summer sowings when germination in depressions was substantially higher than germination on the other microsite types, and least pronounced for spring sowings when seedling numbers on depression and flat microsites were similar and relatively low. Conditions over the course of the experiment were favourable for seedling survival and the rate of mortality was low. The number of seedlings surviving on each microsite type at the end of the experiment is, therefore, likely to be an accurate reflection of the total number of seeds to have germinated.

Table 4.1. Analysis of variance of number of seedlings, logarithm transformed, on field microsites following different seasons of sowing.

Source	*D.F.*	*Sums of Squares*	*F value*	*p*
plot(season)	8	18.56	3.22	0.0016
microsite	2	23.38	16.24	0.0001
season*microsite	6	9.74	2.25	0.0385
error	280	201.60		
Tests using plot(season) as an error term				
season	3	12.54	1.8	0.2247

4.3.2 Glasshouse seed germination

Watering regime, microsite and their interaction were significant in influencing the number of seeds to germinate per subplot (Table 4.2a). In the absence of water stress, germination was similar on the 'flat', 'shallow', 'depression' and 'shade' microsites, while germination on 'clay' and 'hillock' microsites was depressed (Fig. 4.3). By contrast under the intermittent watering regime no germination occurred on the 'flat', 'hillock' or 'clay' microsites and very little on the 'shallow' microsite. Germination was more abundant on the more humid microsites, 'depression' and 'shade', but still poor relative to the continuously watered plots (Fig. 4.3). After the intermittently-watered plots were changed to the continuously watered regime, germination was increased on all microsites. While there was no difference following transfer to continuous watering in the number of seeds to germinate on 'depression' and ' shade' microsites compared with similar microsites that had been continuously watered throughout,

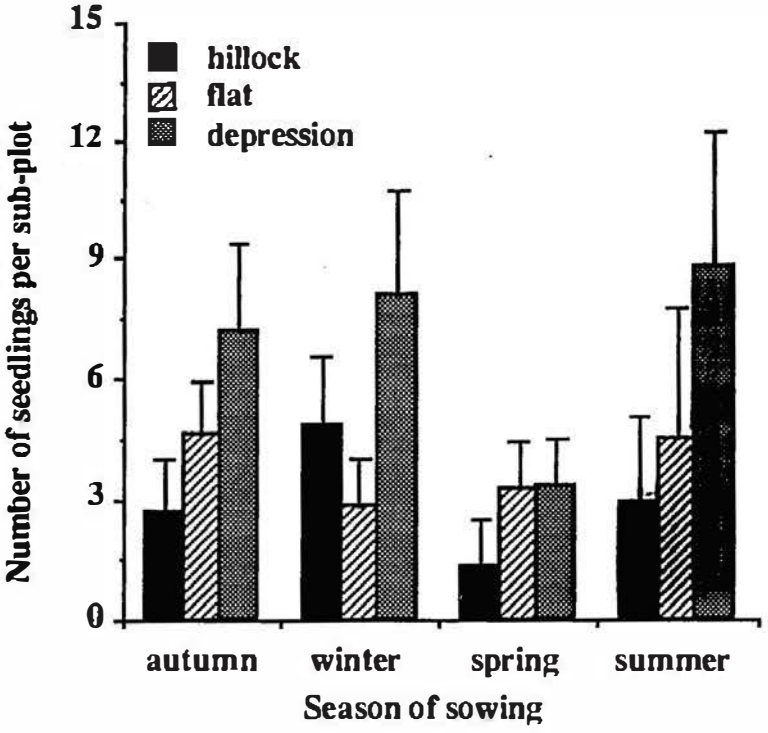


Fig. 4.2. Interaction of season of sowing and microsite on the number of seedlings detected per subplot in the field in autumn of the third year. Detailed demographic studies (see chapter 6) had shown mortality to be slight and numbers detected are an accurate representation of the number to have germinated. Error bars are the 95% confidence interval of the mean.

germination on all other microsites remained depressed (Fig. 4.3, Table 4.2b). Stratification of the remaining ungerminated seeds in the originally intermittently watered regime only resulted in an additional 5% germination. These were not systematically located within microsites and did not change the mean germination number of any of the microsites significantly.

The germination rate on the more protected microsites, 'shade' and 'depression', was significantly more rapid under conditions of continuous watering than for other microsites (Fig. 4.4, Table 4.2c). Due to the low percentage germination it was not possible to calculate germination rates for the intermittent watering regime. However, when this treatment was subjected to continuous watering, germination was rapid, most particularly for seeds in the 'depression' and 'shade' microsites. The germination rate of these seeds was significantly more rapid than seeds which had been continuously watered from the start (Table 4.2c). The ranking of rates of germination was similar however between watering regimes and no interaction between watering regime and microsite on rate of germination was indicated by analysis of variance.

4.3.3 Glasshouse seedling survival

Significant differences in survival times were observed between microsite and age combinations (Table 4.3). Older seedlings were generally more resilient to conditions of declining soil moisture than were younger seedlings (Fig. 4.5, Table 4.4), although seedlings in all age classes survived for long periods without watering. Mortality was generally low until soils had dried to a critical level, after which mortality was rapid, although young seedlings on microsites which impeded root penetration, the 'clay' and 'shallow' microsites, displayed two phases of mortality. The first of these was associated with the death of individuals that failed to establish good root contact, most particularly in the case of 'clay' microsites where radicles were observed to meander over the soil surface.

While older seedlings generally had a longer mean survival time than younger seedlings this was to some extent mediated by microsite. The older seedlings on the harshest microsite, 'shallow', survived for less time than seedlings in the intermediate age class on all but 'clay' and 'shallow' microsites. Survival of the youngest age class seedlings on the most humid microsite, 'depression', was equivalent to survival of the 'oldest' age class on the 'shallow' microsite and the intermediate age class on the 'hillock' microsite. The differentiation of survival times by microsite was least marked for the oldest seedlings and most marked for the youngest seedlings. It was only the 'shallow' microsite that significantly

shortened mean survival time of old seedlings. The intermediate age class of seedlings on 'flat', 'depression', and 'shade' microsites survived significantly longer than seedlings of that age class on 'clay' or 'shallow' microsites. Survival of the youngest age class, however, was significantly longer on 'depression' microsites than any other microsite type, and survival on 'shade' and 'flat' microsites, was superior to 'clay' and 'shallow' microsites (Table 4.4).

Table 4.2. Analysis of variance of effect of watering regime and microsite on number of seeds to germinate and on germination rate.

a. Germination number prior to continuous watering of intermittent watering treatment

Source	*D.F.*	*Sums of Squares*	*F value*	*p*
water*microsite	5	161.25	5.49	0.0002
row*column*microsite	20	93.22	0.79	0.7159
error	100	587.64		
*Tests using row*column*site as an error term*				
row	5	17.06	0.73	0.6080
column	5	35.06	1.50	0.2330
microsite	5	368.06	15.79	0.0001
error	20	93.22		
Tests using plot(water) as an error term				
water	1	2162.25	23.46	0.0401
error	2	184.36		

b. Germination number after continuous watering of intermittent watering treatment

Source	*D.F.*	*Sums of Squares*	*F value*	*p*
water*microsite	5	251.37	4.66	0.0007
row*column*microsite	20	128.75	0.60	0.9064
error	100	1077.97		
*Tests using row*column*site as an error term*				
row	5	22.39	0.70	0.6327
column	5	54.56	1.70	0.1818
microsite	5	909.97	28.27	0.0001
error	20	128.75		
Tests using plot(water) as an error term				
water	1	480.34	4.98	0.1554
error	2	193.07		

c. Germination rate (time to 50% germination) after continuous watering of intermittent watering treatment

Source	*D.F.*	*Sums of Squares*	*F value*	*p*
water*microsite	5	17.33	0.57	0.7236
row*column*microsite	20	155.23	1.27	0.2278
error	67	408.31		
*Tests u ing row*column*site as an error term*				
row	5	43.03	1.11	0.3869
column	5	6.89	0.18	0.9679
microsite	5	285.00	7.34	0.0005
error	20	155.23		
Tests using plot(water) as an error term				
water	1	5067.57	12586.80	0.0001
error	2	0.80		

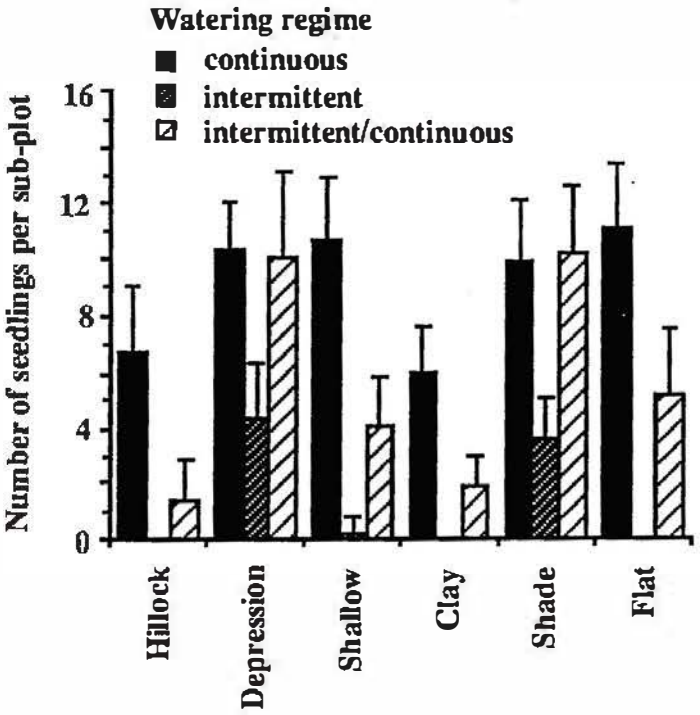


Fig. 4.3. Effect of microsite and watering regime on germination. Watering regimes are continuously watered, intermittently watered and intermittently watered and then transferred to continuous watering. Error bars are the 95% confidence intervals of the mean.

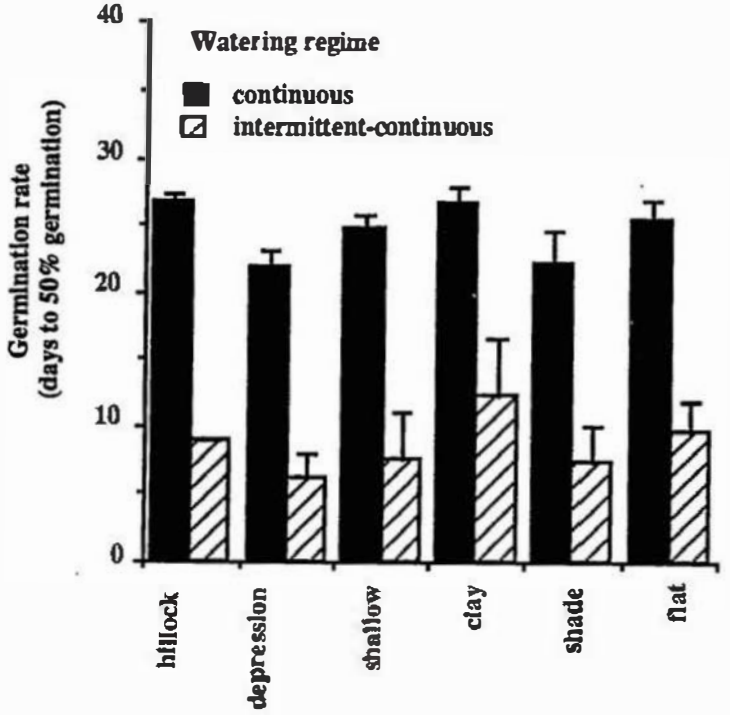


Fig. 4.4. Microsite and watering regime effects of germination rate. Error bars are the 95% confidence interval of the mean.

The covariates of row, column and tray all significantly affected the survival function (Table 4.5), with survival time negatively related to row and positively related to column and tray. That is, rows and columns towards which water drained had a higher survival time. Survival times were slightly longer in tray 2 than tray 1. The significance of these covariates indicates the sensitivity of survival times to small changes in soil conditions.

Table 4.3. Test of equality of survival curves of age by microsite combinations, using the approximate chi-square statistic for the log-rank test and the Wilcoxon test.

Test	*Chi-Square*	*D.F.*	*p**
Log-Rank	240.1	17	0.0001
Wilcoxon	230.6	17	0.0001

* This is an approximate probability based on distributional assumptions (see SAS 1989 for details).

Table 4.4. Effects of age and microsite on mean survival time. Bracketed figures are the standard error of mean survival time. Like letters indicate groupings of survival times at the 95% confidence interval.

Age	*Microsite*	*Mean Survival Time (days)*	*Mean Survival Time Groups*
old	depression	84.4 (1.2)	A
old	flat	83.1 (2.1)	AB
old	clay	81.0 (3.5)	ABCD
old	shade	82.2 (1.9)	ABCD
old	hillock	79.9 (3.3)	ABCD
intermediate	flat	79.4 (1.9)	BCD
intermediate	depression	77.9 (1.6)	D
intermediate	shade	76.1 (2.3)	DE
intermediate	hillock	75.8 (2.1)	DEF
old	shallow	72.2 (3.8)	EF
young	depression	70.7 (3.3)	EF
intermediate	clay	69.8 (3.9)	F
young	shade	62.2 (2.8)	G
young	flat	59.5 (4.6)	GH
young	hillock	56.9 (5.0)	GHIJ
intermediate	shallow	51.8 (3.6)	HIJ
young	clay	48.4 (6.4)	IJK
young	shallow	39.3 (2.7)	K

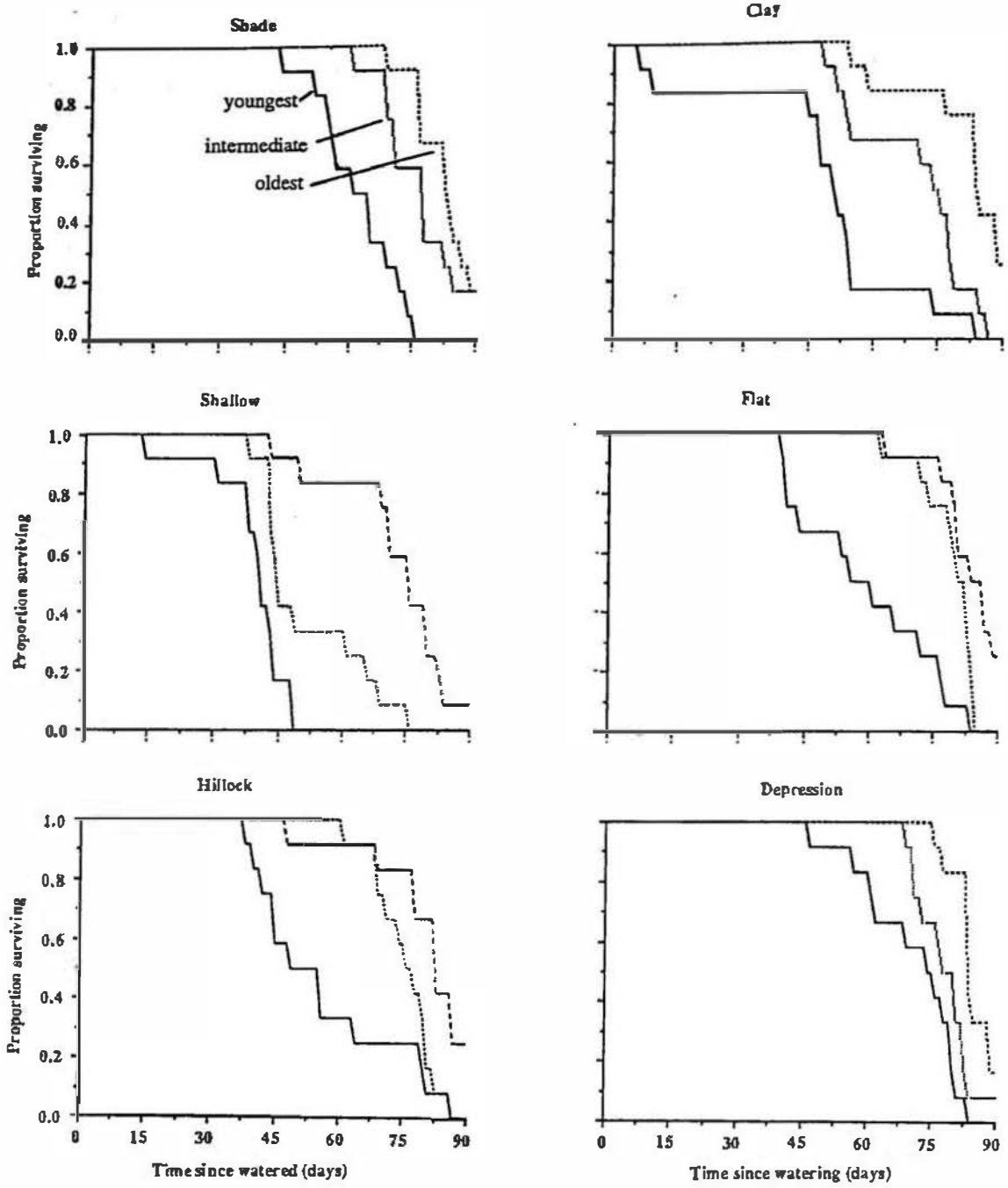


Fig. 4.5. Microsite and age effects on the distribution of survival times under conditions of sustained drought. Key to ages: Youngest ——— ; Intermediate ·····; Eldest - - - - -.

Table 4.5. Univariate Chi-squares for the Wilcoxon test for association of response with covariates pooled over treatment strata.

Variable	*Wilcoxon statistic*	*Variance*	*Chi-square*	*Probability*
row	-53.8	190.5	15.2	0.0001
column	37.2	188.0	7.4	0.0066
tray	10.2	16.5	6.3	0.0118

4.3.4 Seed and seedling characteristics

The leaf diffusive resistance of a seedling under water stress was independent of the ability of the seed it originated from to germinate under water stress (Fig. 4.6, Table 4.6). In so far as leaf diffusive resistance under conditions of low soil water availability can be considered a discriminatory characteristic of seedling drought tolerance, and the ability to germinate under moisture stress a measure of seed drought tolerance, there does not appear to be any correlation between the attributes of seed and seedling drought tolerance.

Table 4.6. Repeated measures analysis of variance of seedling transpiration rate.

Source	*D.F.*	*Sum of Squares*	*F value*	*p*
Univariate tests of hypothesies for within subject effects				
stress level	2	19.61	142.45	0.0001
stress level*pot	34	23.22	9.92	0.0001
stuess level*seed origin	4	0.25	0.93	0.4565
error (time)	32	2.21		
Univariate test for between subject effects				
seed origin	2	0.22	1.55	0.2418
pot	17	24.43	19.91	0.0001
error	16	1.16		

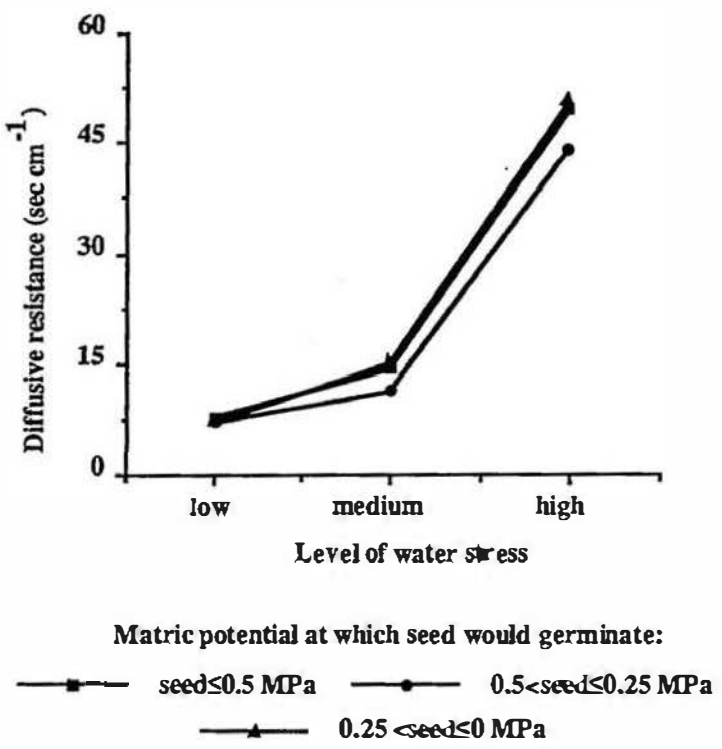


Fig. 4.6. Transpiration rate of seedlings arising from seeds able to germinate under differing levels of osmotically applied moisture stress.

4.4 Discussion

Small differences in microtopography in the field were observed to have a pronounced influence on the spatial distribution of seedlings. Seedling frequency was significantly higher in depressions than on flat terrain or on hillocks. This may have arisen because these sites are better for germination, or because seed wash increased the number of seeds in these areas. While the latter is undoubtedly a partial contributor to the observed result, it is probably minor since the seed of *E. delegatensis* is small in relation to the surface roughness of freshly prepared soil, and seeds were observed to quickly settle into the surface soil matrix. Further, when plot boundaries straddled depressions very little seed was observed to germinate outside the plot, indicating that seed movement had been slight. The effect of microtopography was influenced by the season in which seed was sown, and hence the season in which the majority of seed germinated. Seed sown in winter germinated predominantly in late winter and early spring; seed sown in spring germinated in late spring; seed sown in summer germinated in early autumn and seed sown in autumn germinated in late autumn, early winter and early spring. Clearly the weather in which seeds are germinating, and hence conditions at the soil surface, and the age of newly emergent seedlings when confronted with climatic adversity will vary with the time of sowing. For example, spring-sown seed was clearly the least successful overall. Presumably at this time there was a delay in germination due to dry conditions, even in depressions, in the months following sowing. Further, some seed would not have been able to germinate until following stratification over winter. Mortality of seed during this interval, as a result of the combined actions of insect harvesting, fungal attack and induction of secondary dormancy, may have greatly reduced that available for seedling establishment.

Seeds sown in the summer showed the most marked differentiation in establishment by microsite. Of the microsite types examined, depressions resulted in the greatest seedling numbers, followed by flat terrain with hillocks resulting in the least. It was demonstrated in the glasshouse that under regimes of intermittent watering, germination was restricted to the most protected, and hence probably more humid, microsites. Seeds in exposed microsites were killed, rather than merely becoming dormant, as demonstrated by the lack of germination following prolonged stratification. Approximately 50% of the seed sown was dormant, and this result indicates that dormant seed may be as vulnerable as non-dormant seed to dehydration after being held imbibed close to germination for a reasonable period of time. It was found in Chapter 2 that *E. delegatensis* becomes

increasingly susceptible to dehydration if imbibition proceeds longer than 60 hours. Death in the field probably resulted from dehydration after germination had progressed to an irreversible stage. In the glasshouse, seeds in protected niches survived, possibly because dehydration was averted between watering events. These seeds were able to germinate rapidly when transferred to continuously watered conditions (Fig. 4.4). Protected microsites probably facilitate germination under water limiting conditions by allowing water uptake due to increased humidity in the immediate seed environment, and by preventing water loss in intervening dry periods. Seeds on these microsites may, therefore, be able to exploit short periods of wet weather.

Under conditions of low water stress in the glasshouse the importance of microsite was negligible. Only on the most exposed microsite, 'hillock', and on the microsite 'clay' where soil texture may have prevented good seed-soil contact, was germination inhibited. This is in agreement with field observations where germination on rises was highest relative to other sowing times following winter sowings when it is likely that seeds germinated under the least moisture stress.

Microsite requirements for seed germination and seedling survival may differ, and indeed may change with seedling age. The restriction of the soil profile depth, the 'shallow' microsite in the glasshouse experiment, did not affect germination relative to the 'flat', or control, microsite. Survival times of seedlings on such microsites under conditions of drying soil, however, were severely truncated. As seedlings grow and are capable of exploiting environments beyond that affecting germination, they become less sensitive to microsites. In this work, the differentiation of survival times during prolonged drought was highest for the youngest seedlings and least for the oldest seedlings.

In this study the ability of a seed to germinate under stress was not correlated with the leaf diffusive resistance of the subsequent seedling when subject to water stress. Water stress resistance and tolerance in plants can be manifest in many physiological responses (e.g. Kozlowski 1976) and it may be that the simple index of stress tolerance used in this work has missed subtle differences. While it might be anticipated that many seed and seedling characteristics would be correlated, this is only well documented for seed size and subsequent plant vigour (Harper and Obeid 1967; Schaal 1984; Tripathi and Khan 1990). Even in this case a strong genetic correlation may not exist since seed size is frequently strongly influenced by non-genetic maternal effects rather than genetic causes (Schaal 1984). However, at the provenance level seed characteristics are

frequently correlated with seedling characteristics. For example, the dormancy level of *E. delegatensis* seed (Boland and Dunn 1985) is correlated with provenance differences in frost tolerance (Hallam and Reid 1989). However, this does not mean that there is a correlation between individual seed dormancy and seedling frost tolerance. Selection at the time of germination for *E. delegatensis* is high with perhaps only one seed out of 50 establishing as a seedling (Lockett 1991). However, before a tree reproduces perhaps only one out of 100 seedlings will remain (Campbell and Bray 1987). At the time of this latter selection the tree will have grown well beyond the limits of the seed microenvironment. Indeed this was already evident in the decreased influence of microsite on the survival of seedlings only weeks different in age reported in this study (Fig. 4.5). This difference in the grain of microsite variation that affects seed germination characteristics compared to that which affects seedling survival may mean that within provenances selection will not occur for related seed and seedling characteristics. Similarities at the provenance level may thus be a result of parallel selection for traits rather than any causative link as a result of microsite selection at the germination stage.

In conclusion, small scale variation in soil conditions, at the scale of tens of centimetres, affected the germination and establishment of *E. delegatensis* seeds and seedlings. The importance of this variability in microtopography was strongly influenced by season and the level of environmental stress, and its importance was diminished as seedlings aged. Because of the different requirements for seed germination and seedling growth a favourable microsite for germination was not necessarily a favourable site for seedling survival. In *E. delegatensis*, at least, selection due to microsite differences at the time of germination may not impact on the developmental characteristics of the seedlings.

Variation in the germination niche at the micro-scale may be as great as changes in average conditions over the whole range of a species. In this study very small modifications to the soil surface changed the germination of viable, non-dormant seed from 0 to 100%. The few data available on spatial heterogeneity in forests (Lechowicz and Bell 1991; Bell and Lechowicz 1991) suggest that forest floor understorey environments are predictably similar over distances of a few metres. In determining plant distribution, spatial heterogeneity may need to be considered over a number of scales, from that at the micro-scale of tens of centimetres to that of the macro-scale over which physical patterns such as rainfall change. While the latter scale is likely to give rise to the correlated selection of seed germination and seedling characteristics, the former may cause disruptive selection of seed

germination characteristics that are uncorrelated with seedling characteristics within a provenance.

Chapter 5: Ontogenetic variation in frost resistance of *Eucalyptus delegatensis* R. T. Baker

5.1 Introduction

The persistence of a species in a given habitat is determined by the most susceptible stage(s) in ontogeny. For many plants it is often the dispersal and seedling establishment phases that are the most critical to reproductive success (Harper 1977). New plantings of *Eucalyptus* are generally at a greater risk of frost damage than older trees, and severe or untimely frost can destroy whole areas of young regeneration (e.g. Cremer 1962; McKimm and Flinn 1979; Griffin *et al.* 1982). Extensive damage to older trees is rarer, and has been reported only after exceptionally severe frosts (Calder 1850; Bond 1945; Davidson and Reid 1985).

Examination of frost resistance in *Eucalyptus* has predominantly concentrated on variation between well-established plants (e.g. Raymond *et al.* 1986; Hallam and Reid 1989), or variation between seedlings of the same age (e.g. Rook *et al.* 1980). Tibbits (1986), however, demonstrated increases in frost resistance between saplings one, two and five years old. Cremer and Mucha (1985), approaching the question from the other end of the ontogenetic range, found that dry seed is more resilient to freezing than is imbibed seed, and imbibed seeds more resistant to frost than germinating seed. Between these stages little is known of ontogenetic variation in frost tolerance. Paton (1981) found that the frost sensitivity, and the ability to harden, of tissue from six month old seedlings, was related to the leaf node of origin. This is highly suggestive that the frost resistance of seedlings will change rapidly as they age during the first few months of life. However, differences in frost damage to different-sized seedlings in the field have in the past been ascribed to taller seedlings being above the coldest layer of air (Meskimen 1983; Tibbits 1986).

The extent of damage to a seedling from a particular frost event may, therefore, depend on the interaction of height, genotype, leaf node and age. While the influence of genotype on frost resistance has received much study (e.g. Tibbits and Reid 1987; Hallam and Reid 1989), and the effect of cold air layering is well documented (e.g. Davidson and Reid 1985), the change in the frost sensitivity of

tissue from a given node with age, and the effect of this on overall seedling frost resistance, has received less attention. Such changes have important implications for the artificial establishment of eucalypts, since the demographic structures of the population at the time of frost events may determine the success or failure of reafforestation efforts. The fate of individual cohorts of seedlings will clearly determine the success or failure of a particular sowing time. A foreknowledge of changes in frost resistance with seedling age, coupled with known likelihoods of timing of frost events, may indicate the risks associated with certain sowing times.

This chapter reports on an experiment in which the frost resistance of leaves from different nodes of seedlings of four ages is compared.

5.2 Methods

Seed of *E. delegatensis*, (E00080, M36 Provenance, see Chapter 1 for details) was sown on four separate occasions onto trays of potting mix. At the cotyledon stage seedlings were pricked into individual pots containing potting mix. The sowings gave rise to four cohorts of seedlings. The eldest were approximately six months old. These seedlings often had several leaders, with the leaves on the twelfth leaf node partially expanded, and the thirteenth leaf pair visible. Cotyledons had been shed, but the first leaf pair was still retained. The second eldest class of seedlings were approximately two months old. These seedlings had the first and second leaf pairs fully expanded, and the third leaf pair visible. Cotyledons were retained on most seedlings. The next eldest cohort consisted of seedlings approximately one month old. The first leaf pair on these seedlings was still not fully expanded and the second leaf pair was just visible. Cotyledons were retained on all seedlings. The youngest cohort, approximately two weeks old, were newly emerged seedlings possessing only cotyledons. All seedling were hardened for four weeks in a cool room at 5°C under a mixed incandescent-fluorescent light source (≈ 200 μmol m^{-2} s^{-1} at plant height) with an eight h photoperiod. In all, eight tissue types were identified for testing: cotyledons from cotyledonary stage seedlings, one month and two month old seedlings, the first leaf pair from seedlings one, two and six months old, the second leaf pair from seedlings two months old and the twelfth leaf pair from seedlings six months old.

Frost resistance of material was assessed using the electrical conductivity of diffusate method of Hallam and Tibbits (1988). Because cotyledons were small, a minor modification to the technique was required. Instead of disks being taken of

test tissue, each sample consisted of four 0.25 cm^2 squares of leaf tissue; 0.25 cm^2 square being the largest piece of material obtainable from a cotyledon. Material from different plants was randomised between temperature treatments and samples were lowered to -1, -2, -3.5, -5 or -7°C in an air filled chamber (Hallam and Tibbits 1988). A minimum of six replicates was used for each tissue type at each temperature.

5.3 Results

There were significant differences in frost damage, as measured by electrolyte leakage, between tissues from seedlings of different age ($P<0.001$), tissues collected from different leaf nodes ($P<0.001$) and leaves at different stages of expansion ($P<0.01$) (Fig. 5.1). Seedlings at the cotyledon stage were the most frost sensitive tested, having a mean lethal temperature (the temperature at which 50% loss of cellular electrolytes resulted, Hallam and Tibbits 1988) of approximately -1.5°C, compared with -2°C for seedlings one month old, -2.5°C for seedlings two months old and -3.5°C for seedlings six months old (Fig 5.1a). Material from the cotyledonary node was the most frost sensitive, irrespective of the age of plant from which it was sampled (Fig. 5.1b). Material from the newly-expanding twelfth leaf node on seedlings six months old was less frost sensitive than material from the cotyledonary node, but still significantly more sensitive than material from nodes one and nodes two (Fig. 5.1b). While there was no interaction between node and age ($P>0.1$) on the frost sensitivity of leaf tissue in the two-way analysis of variance, material from leaves that were not yet fully expanded, the twelfth leaf node on seedlings six months old, the second leaf node on seedlings two months old and the first leaf node of seedlings one month old, was significantly more sensitive than more fully expanded leaves ($P<0.05$), with mean lethal temperatures of -2.5°C and -3.5 °C respectively (Fig. 5.1c).

Combining these results with the results of Cremer and Mucha (1985) it is possible to develop a generalised overview of the change in frost sensitivity of *E. delegatensis* in the first few months of development (Fig. 5.2). Because of the differences in provenance and potential differences in hardening regime, caution needs to exercised in combining these results. Nevertheless it can be seen that in both studies the most susceptible life stage of *E. delegatensis* to frost appears to be at, or immediately following, germination.

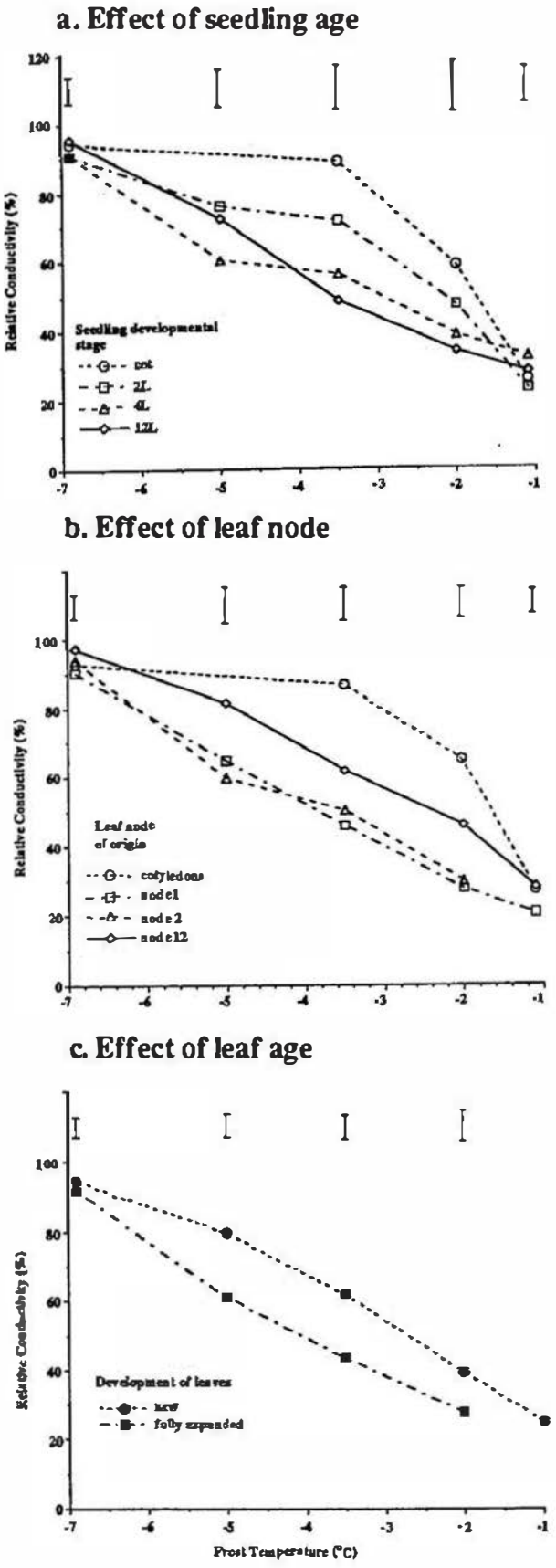


Fig. 5.1. Effect of seedling age, leaf node of origin and leaf developmental stage on relative tissue damage at different frost temperatures. Error bars are the least significant differences (95% C.I.) for pairwise comparison. In (a) nodes are pooled within ages, in (b) ages are pooled within nodes and in (c) 12 th node from 12 leaf pair stage seedlings, 2nd node from 4 leaf pair seedlings and 1st node from 2 leaf pair seedlings are pooled in the 'new' class.

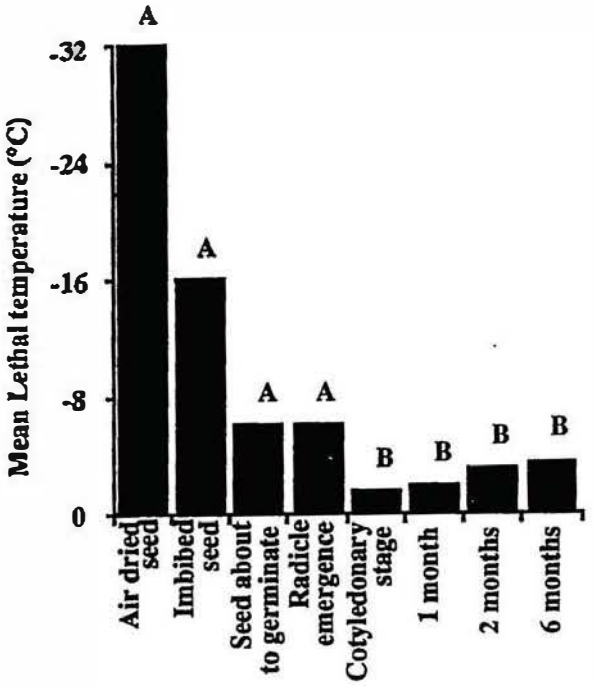


Fig. 5.2. Changes in frost hardiness of *E. delegatensis* during early development. Sources: A=Cremer and Mucha 1985; B=this work.

5.4 Discussion

The change in frost sensitivity of *E. delegatensis* with age found in this study was similar to the pattern observed in other plants (Cary 1975; Fuller and Eagles 1978). Frost tolerance increased with seedling age up to two months of age, or until the first two leaf pairs were expanded, after which only a slight change was evident. Hallam (1986) working with more advanced seedlings (600-800 mm tall, or approximately twice the height of the seedlings six months old used in this study) from a similar provenance and hardened for four weeks at 4°C, found a similar mean lethal temperature of -3.5°C suggesting that further gains in frost resistance as plants age will be slow relative to those made in the first months of development. The change in mean lethal temperature from -1° to -3.5°C over the first six months of growth, is comparable to the change in frost tolerance observed by Tibbits (1986) when comparing one and five year old *E. nitens* plants. Since the interaction of seedling age and leaf node sensitivity was not significant, the

change in sensitivity with age may be largely a result of the increasing proportion of more frost tolerant leaves on older plants. Nevertheless, when fully expanded leaves were compared to partially expanded leaves it was apparent that leaf age was affecting the frost tolerance of tissue. In addition to the effects of genotype and seedling height with respect to cold air layering, therefore, the amount of damage suffered by an individual will be affected by its stage of development and by the amount of new growth it displays.

Eucalyptus delegatensis is most susceptible to frost as a newly emergent seedling (Fig. 5.2). Whilst newly emergent leaves on older plants will also be susceptible to relatively mild frosts, extensive tissue damage and death of older plants is likely only at considerably lower temperatures than those resulting in the death of cotyledons. Cotyledonary stage seedlings will be particularly susceptible, not only because of the sensitivity of leaf tissue from the cotyledonary node, but also their position close to the soil surface where temperatures are often lowest. Frost is, therefore, most critical to regeneration success of *E. delegatensis* during the very early stages of seedling establishment.

Chapter 6: Effect of sowing time on germination, survival and growth in the field

6.1 Introduction

Inferences regarding the ecological significance of emergence traits determined in glasshouse or laboratory studies, only become meaningful if they can be demonstrated to influence outcomes in the natural situation. Inferences from field experiments alone are frequently "weak" since alternative explanations of phenomena are frequently available (Hairston 1989). The corroboration of glasshouse experiments by field studies, however, gives confidence that results are applicable in the real world.

Optimising the sowing time of seed in the field is an applied question in plant demography. A knowledge of the numbers and fates of individuals is necessary if useful inferences are to be drawn from field experiments which involve multiple sowing dates. But even high quality demographic data is of limited use in the absence of correlation with environmental conditions since causal and casual factors cannot be separated. Where such data is collected simultaneously, and the response of germination to environmental cues is understood, useful ecological observations can be made (e.g. Popay and Roberts 1970).

This chapter details a demographic study in which the emergence and survival of seedlings of *Eucalyptus delegatensis* and *E. amygdalina* are followed. The experiment is similar in design to other eucalypt time of sowing experiments (see Chapter 1). However unlike many of these studies, detailed site weather records were collected. By interpreting the results of different sowing times in the field in terms of the responses of seeds and seedlings to the environmental determinants of germination and survival determined from laboratory studies respectively, some inferences regarding the processes affecting seedling establishment can be made. The outcome from each sowing time provides a separate, although not entirely independent, test for subsequent predictive modelling of emergence.

6.2 Materials

6.2.1 The study sites

The experiment was replicated at two climatically different, but geographically close sites on the east coast of Tasmania, Australia. The first site at Mount Connection, logging coupe MC31d (Plate 6.1), was located near Lake Leake inland from Swansea (55GEP668429). The second, logging coupe BI25b (Plate 6.2), was inland from Bicheno, near Lynes Sugarloaf (55GEP932645) (Figure 6.1). At the Mount Connection site, nearby forest consisted of *E. delegatensis* over an open understorey of *Banksia marginata* Cav. and *Acacia dealbata* Link with a ground cover dominated by a mixture of grasses, sags and sedges grading into *E. pauciflora* Sieb. ex Spreng woodland below the study site. At the Bicheno site *E. delegatensis* was present only on nearby hill tops. The forest near the experimental site consisted of *Eucalyptus obliqua* L'Herit. and *Eucalyptus amygdalina* Labill. open forest over a ground strata consisting of low open heath over a dense litter layer (80% cover) with scattered herbs. Species list for each site are given in Appendix 2.

The Mount Connection site was at an altitude of 540m. The underlying geology was Jurassic dolerite, which had given rise to a yellow-brown silty clay (10YR 3/2 grading to 10YR 6/3), in excess of 75 cm in depth, in which was embedded large dolerite rocks. The Bicheno site was at an altitude of 350m. The underlying geology was also Jurassic dolerite which had given rise to a gradational soil with over 80% of the surface covered by gravel. The top 10 cm was a grey-brown clayey loam (10YR 3/4) grading to white-yellow clay (10YR 6/8) at 60 cm. Both of the experimental plots were flat and uniform in soil characteristics and drainage.

Table 6.1. Macroclimatic data from BIOCLIM (Busby 1991) for the experimental sites.

Climatic Variable	Mt.Connection	Bicheno
annual mean temperature (°C)	9.0	9.9
maximum temperature of warmest month (°C)	19.3	20.1
minimum temperature of coolest month (°C)	1.3	2.0
mean temperature of wettest quarter (°C)	5.8	8.3
mean temperature of driest quarter (°C)	13.1	14.0
mean annual precipitation (mm)	858	1050
precipitation of wettest month (mm)	87	102
precipitation of driest month (mm)	53	62
wettest quarter precipitation (mm)	245	286
driest quarter precipitation (mm)	173	220

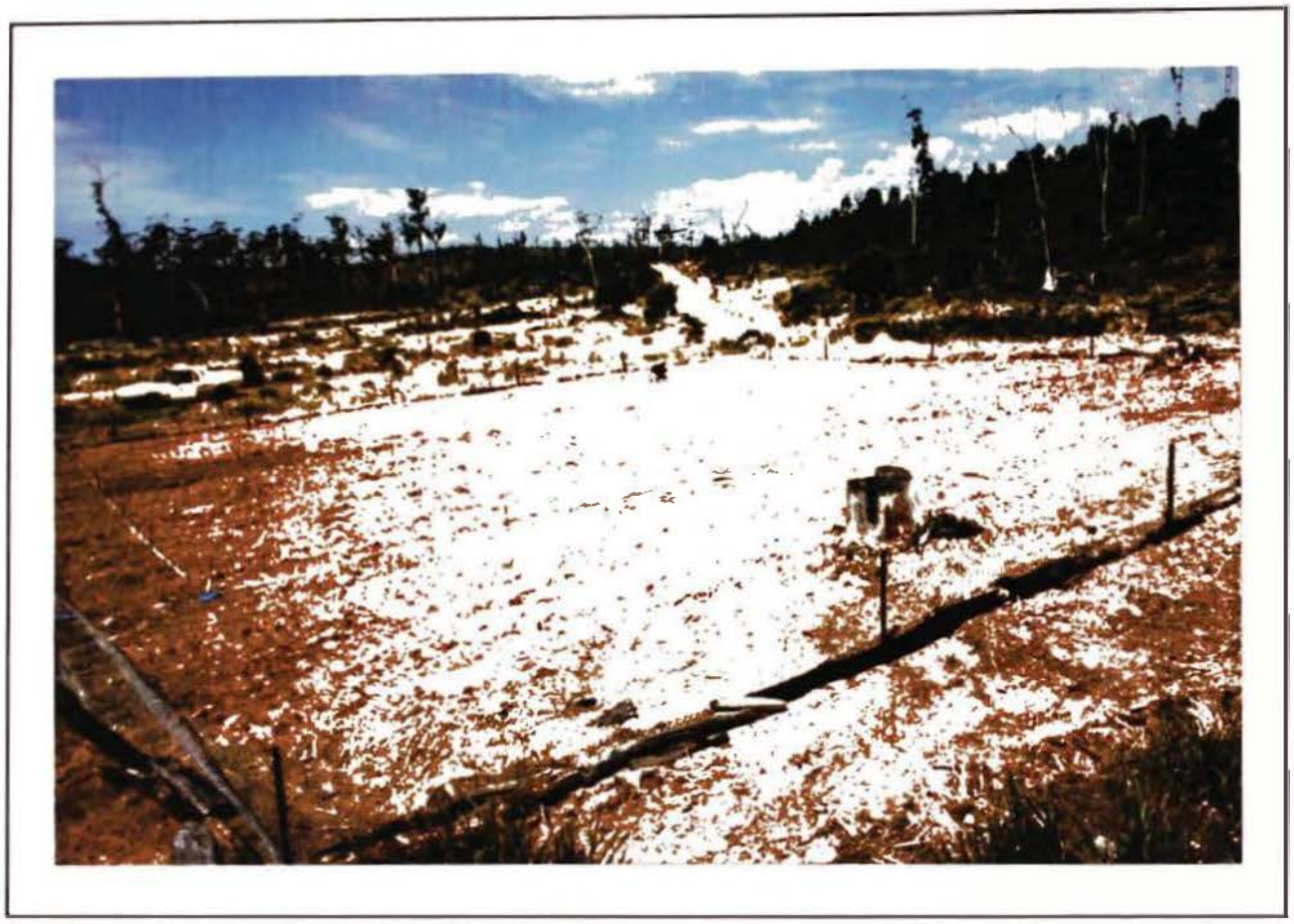


Plate 6.1. Experimental plot, Mount Connection site.

Plate 6.2. Experimental plot, Bicheno site.

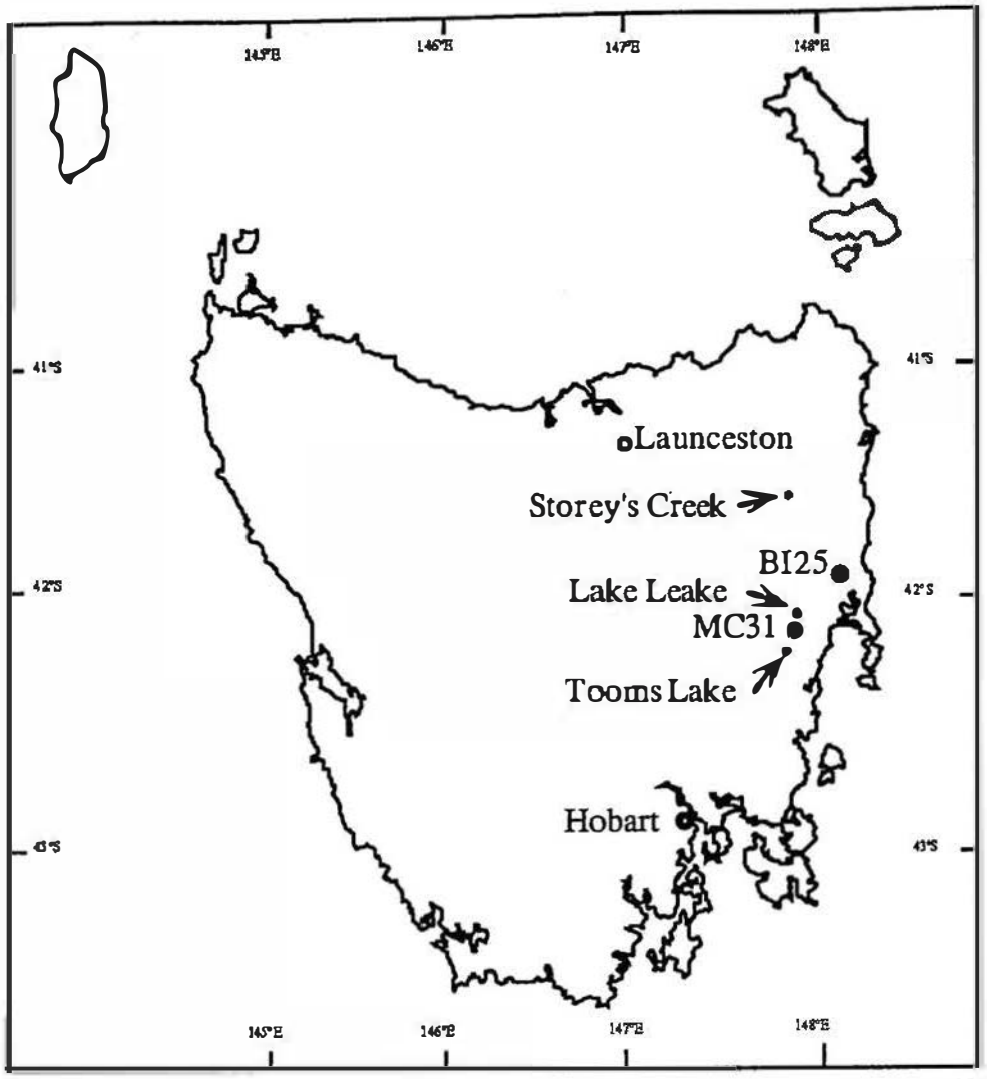


Fig. 6.1. Location of experimental sites and weather station locations referred to in this chapter.

The Lake Leake and Tooms Lake meteorological stations, particularly the former, can be considered to approximate closely the climate at the Mount Connection site. However records do not fully cover the time period of the study. No meteorological station can be considered to describe adequately the Bicheno site. Macroclimatic data from the climate process model BIOCLIM (Busby 1991) for both sites is given in Table 6.1. It can be seen that although the average climatic data differences are slight, the Mount Connection site is cooler and drier, with a relatively dry summer compared to the Bicheno site. The Bicheno site is on a bench on a north-facing slope with good cold air drainage, whereas the Mount Connection site is in the bottom of a broad valley where cold air accumulates. The Mount Connection experimental site is located within the M38 seed zone and

the Bicheno site is located within the M37 seed zone (FCT 1989). Both sites had been clearfelled 3-5 years prior to the commencement of the experiment, and hence it was virtually assured that no dormant seed remained in the ground. Any trees remaining within 200 metres of the experimental plots were felled to remove the possibility of extraneous seed blowing into the plot.

6.2.2 The study seed

Two species were selected for study, *E. delegatensis* and *E. amygdalina.* Both species occurred either at, or near to, the experimental sites prior to logging. Direct gradient analysis (Williams *et al.* in prep) indicates that both species occur abundantly under the average conditions prevailing at the experimental sites. This work suggests, however, that *E. delegatensis* continues to be abundant at average temperatures below those at the experimental sites whereas *E. amygdalina* does not, and that *E. amygdalina* continues to be abundant under conditions drier than the Mount Connection site whereas *E. delegatensis* does not. The *E. amygdalina* seedlot, from the M37 seed zone (FCT 1989) used in the study exhibited no dormancy, germinating completely at 20°C without any prior stratification (92 000 germinants/kg). Two seedlots of *E. delegatensis* were also sown. The first, from M38 seed zone, was selected on the basis of a Forestry Commission, Tasmania (FCT) germination test as a non-dormant seedlot (50 000 germinants/kg without stratification and 47 000 germinants/kg following four weeks stratification at 5°C). The second, from M36 seed zone (M36 seed zone is just north of the Bicheno experimental site), exhibited a high proportion of dormancy (68 000 germinants/kg without stratification and 133 000 germinants/kg following eight weeks stratification at 5°C). This dormant seedlot was sown with and without prior stratification. Stratification involved four weeks moist storage at 5°C. Four weeks stratification left only 10% of seed dormant. To allow this seed to be sown some air drying was necessary (a drying from approximately 45% down to 30% moisture content). Unpublished work by the FCT suggests that this treatment should not affect the dormancy attributes of the seed, although the sketchiness of the reporting prompts care when generalising from these results. The germination characteristics of the two *E. delegatensis* seedlots with respect to temperature, stratification and soil water potential were detailed in Chapter 2.

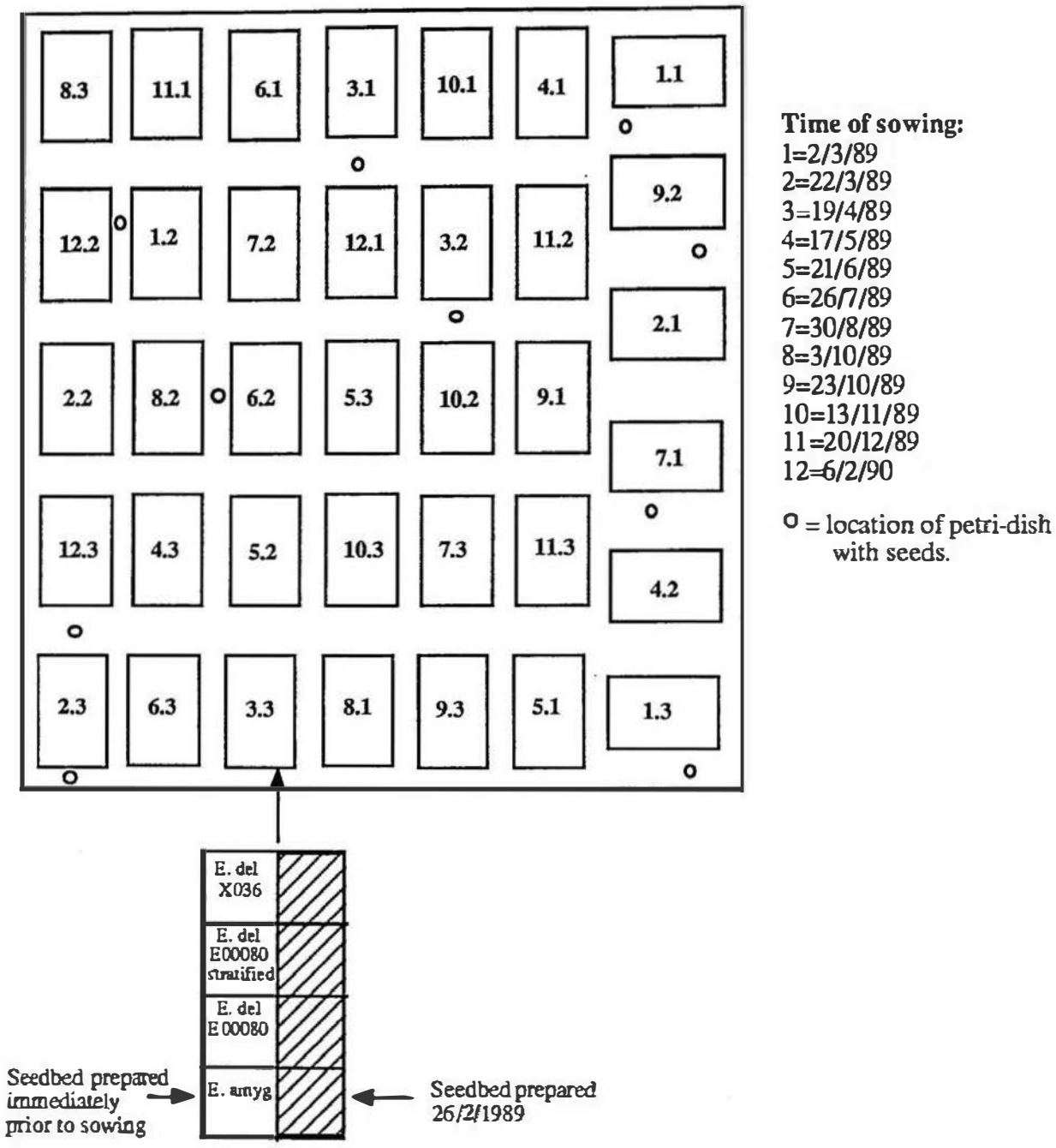


Fig. 6.2. Layout of experimental plots at each field site.

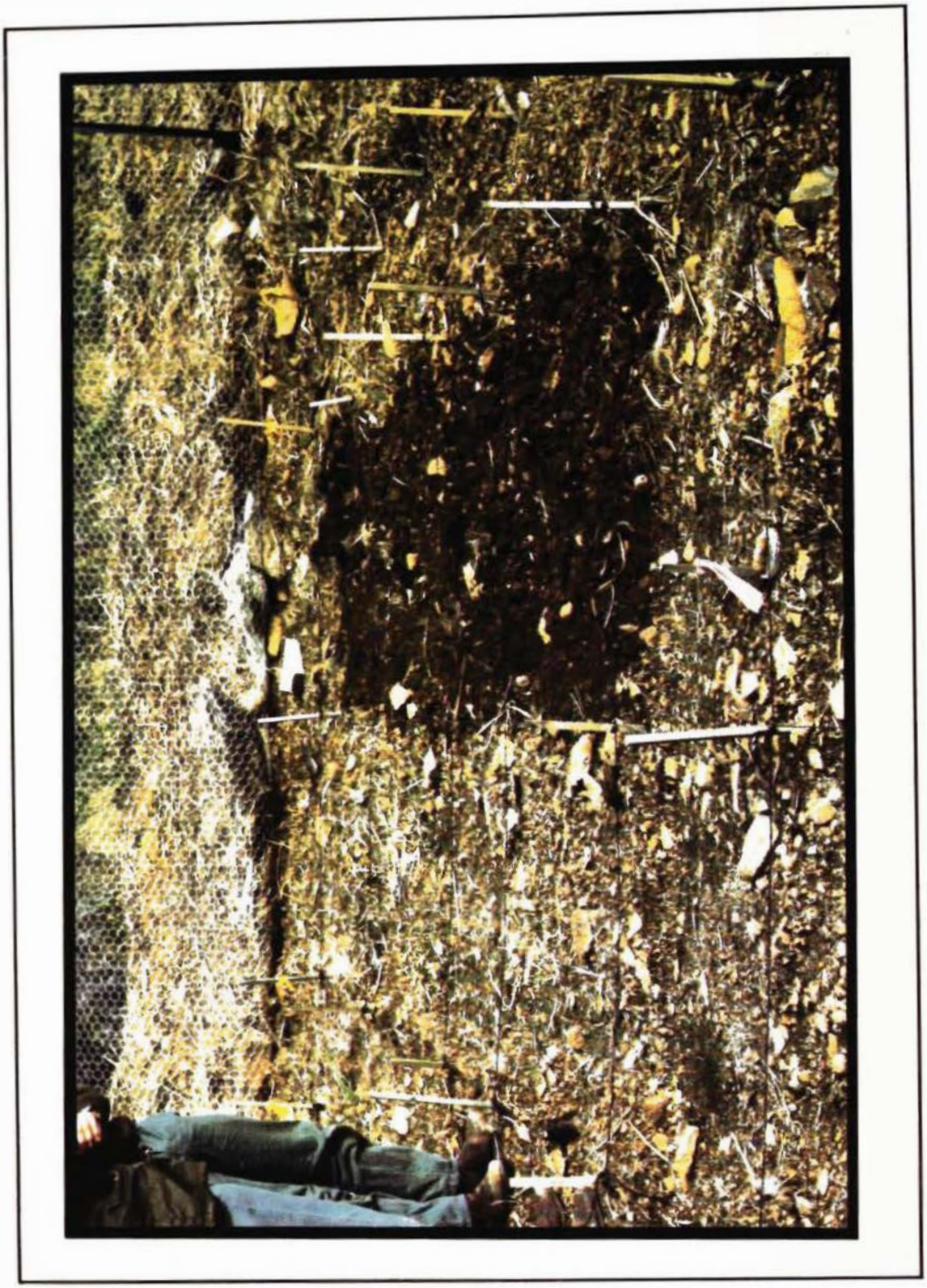


Plate 6.3. Seedbed types used on an experimental plot at the Mt Connection site, 5/10/89. The lefthand side was prepared at the commencement of the experiment on the 27/2/89; the righthand side was prepared at the time the plot was sown, 3/10/89.

6.3 Methods

6.3.1 Experimental Design

The experiment was established as a blocked split plot design. Each site represented a block. Within each block 36 plots were created, comprising three replicates of 12 times of sowing. Each plot was split twice: in the first instance into two seedbed preparation times and in the second into 1 m^2 sub-plots onto which were sown the four differing seedlots. Each plot, comprised of the eight sub-plots, was surrounded by a one metre buffer (Figure 6.2,). The first seed bed preparation time was two weeks prior to the start of the experiment. At this time the vegetation was removed from the whole of the site and the soil cultivated. At the Mount Connection site this was done using a John Deere Loader and at the Bicheno site vegetation was removed by hand and the block rotary hoed. The seedbed on the other half of each plot was given a second preparation by hand-hoeing immediately prior to the sowing of seed (Plate 6.3). Each site was surrounded by a 1.3 m high ringlock fence, with a single strand electric wire on an "outrigger" 20 cm below the top of the fence, to exclude browsing animals.

Seed was applied to each sub-plot using a salt shaker with enlarged holes. Seed was not sown on the outer 10 cm of each sub-plot but was spread uniformly over the remainder. 8.4g of the M38 *E. delegatensis*, 6.7g of the M36 *E. delegatensis* and 2.5g of the *E. amygdalina* was sown, corresponding to 420±60, 900± 20 and 210± 25, seeds respectively. Seed was sown on twelve dates spread over the twelve months following the initial preparation of the seedbed at each site. The times of sowing were most heavily concentrated in spring and autumn when it was presumed conditions for germination would be changing the most rapidly. The sowing dates are given in Fig. 6.2.

6.3.2 Data Collection

Censuses of seedlings were conducted approximately fortnightly for the first twelve months and less frequently over the next fourteen months. Detailed demographic data were collected for the M36 *E. delegatensis* seedlot, both stratified and unstratified, and the *E. amygdalina* seedlot on seedbeds prepared immediately prior to sowing. For these treatments, at each census a colour coded skewer was placed next to each seedling observed since the last scoring (Plate 6.4*a&b*). In this way recruitment and mortality of 25 cohorts were recorded. Germination of seeds sown on to seedbeds prepared at the commencement of the

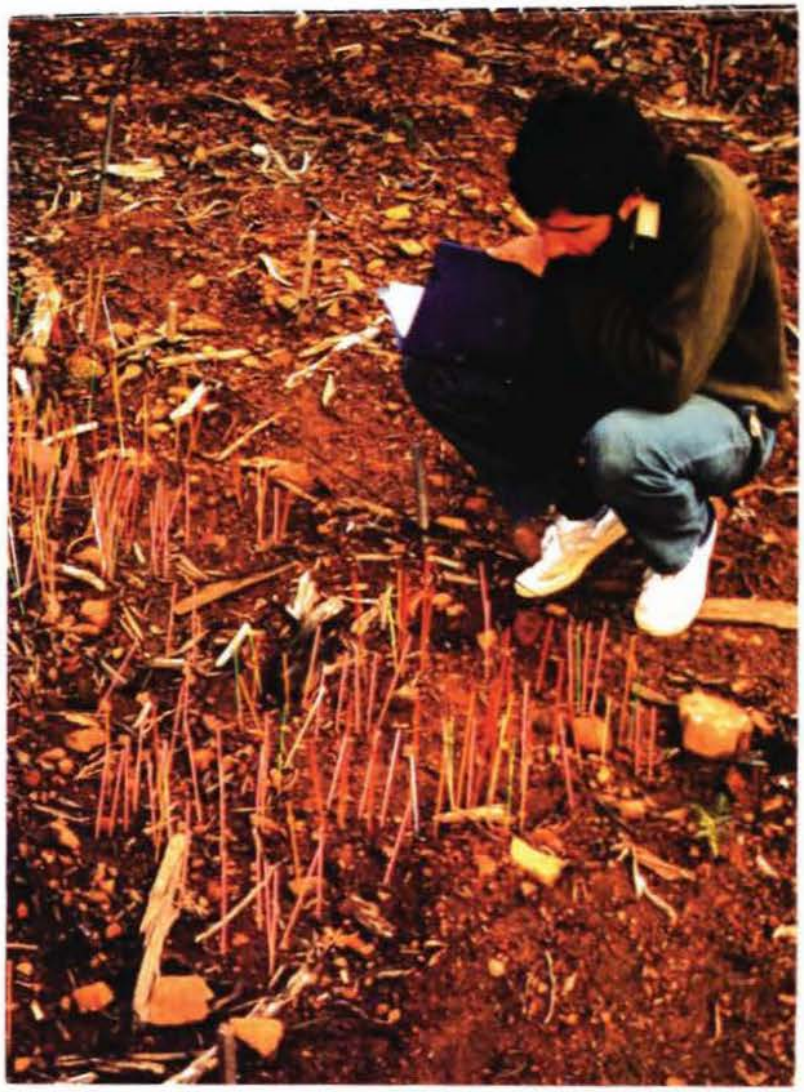
experiment only, and germination of the M38 E. *delegatensis* seedlot were recorded in this way until 18/9/89, and then on the 12/4/90 the number of seedlings present was recorded. At all censuses, seedlings were considered germinated when any evidence of cotyledon emergence was detected. Seedlings were considered dead when 100% of above ground biomass was necrotic. Seedlings recorded as dead were physically removed from the plot if any material remained. Thus, any dead seedlings discovered on a plot at the time of scoring without an adjacent marker were presumed to have germinated and died since the last scoring and allotted the current scoring date as the germination date. Height data were collected on the 18/5/89, 13/11/89,12/4/90, 29/10/90 and 10/4/91 (only BI25 site for last two scoring dates). The height of ten randomly selected seedlings from each age cohort was measured at each scoring.

Seasonal variation in insect predation of seed was measured by laying out 10 baits at each time of sowing. Each bait consisted of a 9 cm diameter plastic petri dish in which two offset entrances had been cut (Plate 6.5). Fifteen apparently viable seeds were placed in each petri dish. The proportion of seeds taken in the first two weeks was recorded

At the Bicheno site, daily temperature maxima and minima, and half hourly recordings of wind speed and rainfall were recorded from the 22/3/89 till 22/6/90 using electronic sensors and a STARLOG® datalogger (Plate 6.6). Equipment failure meant that only the latter two parameters were recorded at the Mount Connection site. Equipment was calibrated before and after installation at the Meteorological Bureau weather station at Battery Point, Hobart. Additionally, at each visit to the sites, maximum and minimum temperatures from a max.-min. thermometer, rainfall from a rain-gauge since the last visit, and soil water potential at time of visiting using a dewpoint microvoltmeter (Wescor 33-RT), were recorded. Weather readings were taken within 10 metres of experimental plots. Soil samples for soil moisture estimates were collected from randomly located positions within the experimental plots.

Temperature estimates for the Mount Connection site were made by using a nearby, and climatically similar meteorological station at Lake Leake (Fig. 6.1). This station, however, only collected data from October 1989 onwards and it was necessary to establish the relationship between this station and the next most comparable East Coast climatic station at Storey's Creek (Fig. 6.1) to complete the coverage of temperature data.

Plate 6.4. Seedlings were marked with painted skewers, the colour of which indicated the date of detection.



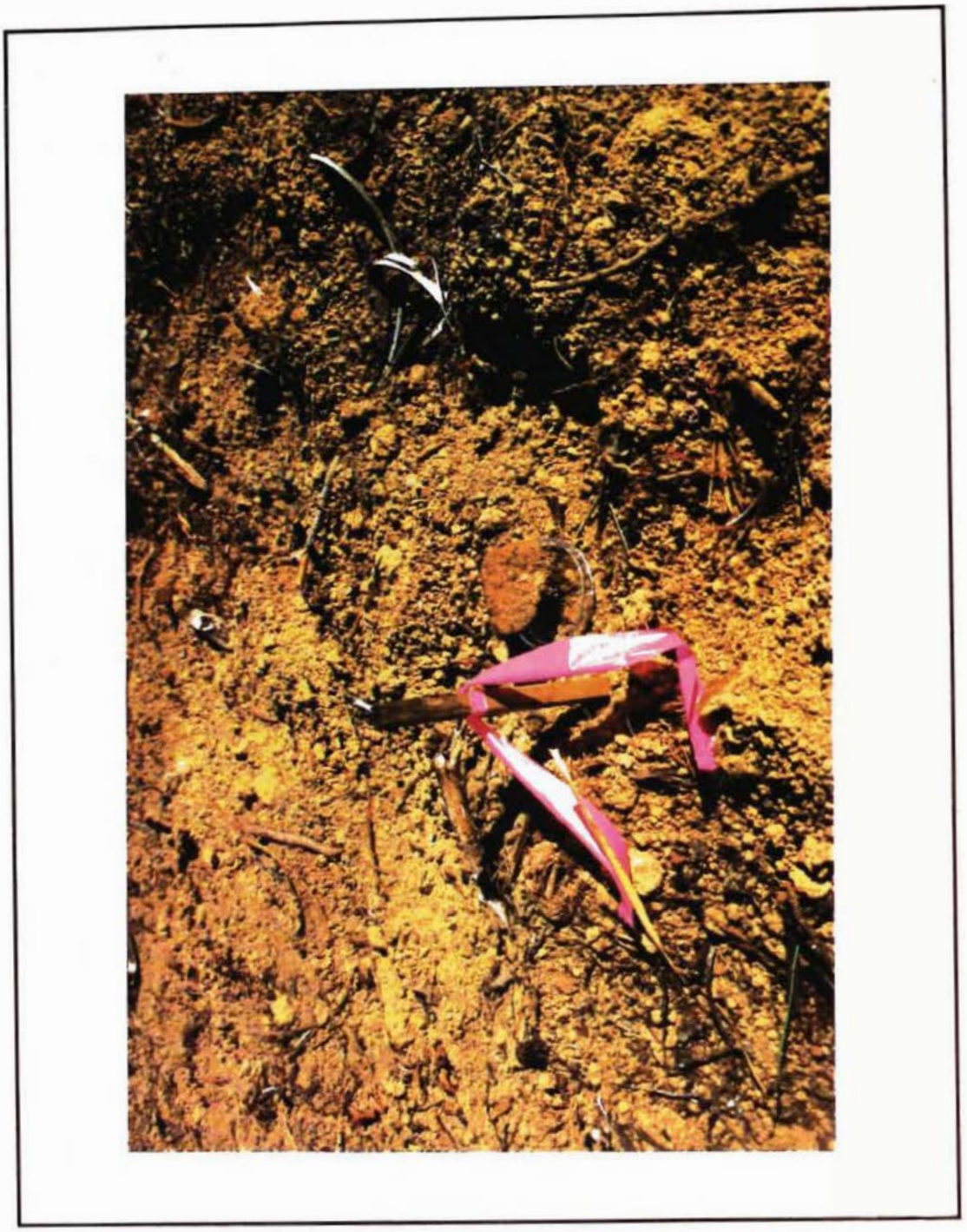


Plate 6.5. View of petri dish in which seed baits were laid to monitor seed harvesting.

Plate 6.6. Automatic weather station at the Bicheno experimental site.

Daily temperature maxima and minima at Lake Leake (LMAX and LMIN) were related to those at Storey's Creek (SMAX and SMIN) by the following equations:

$$LMAX=1.6458+0.8799.SMAX \qquad r^2=0.8563$$
$$LMIN=-2.62+1.6413.SMIN-0.0426.SMIN^2 \qquad r^2=0.6750$$

Soil surface temperatures and soil moisture were calculated using a physically based numerical model outlined in Chapter 8.

6.3.3 Data analysis

Analysis of variance of germination data were conducted using the GLM module of SAS/STAT (SAS 1989). The type III sums of squares was used in the calculation of the *F* statistic because of the unbalanced design. Because in many instances residual analysis indicated the data to be heteroscedastic, the logarithm of the dependant variables was used in analysis (McPherson 1990). Seasonal patterns in the intensity of seed harvesting were analysed using a repeated measures analysis of variance design (SAS/STAT procedure GLM, REPEATED option).

The ratio of germination on recently prepared, compared to that on old seedbed was used in a regression modelling approach to examine the effects of time since seedbed preparation on germination success. A logarithmic transformation of the ratio was made to normalise the residuals (McPherson 1990). Implicit in the logarithmic model is the assumption that the ratio is expected to increase exponentially with elapsed time *(t)*, leading to the regression equation: $ratio=exp^{A+B*t}$. To establish if there is a relation between the ratio and the elapsed time, two models must be compared, model M_1 which contains the equation $M_n=exp^A$ and model M_2 which contains the equation $M_n=exp^{A+B*t}$. This is indicated by the probability that *B* is significantly different to zero. It is also implicit in the hypothesis that if the elapsed time equals zero then the ratio will equal one, and hence the log of the ratio will equal zero. This can be tested by observing whether *A* is significantly different to zero.

Logistic analyses were used to examine the interactions of species, season, age and site on the probability of surviving from one census interval to the next. Survival within any one time period is a binary response variable; a seedling either survives or it does not. Such variables are binomially distributed and survival rate can be analysed by transforming probabilities to generalized logits

and carrying out multivariate frequency analysis using the SAS/STAT procedure CATMOD (SAS 1989). Because data-pooling was necessary to ensure adequate observations per cell (>5), a simplified set of explanatory variables was used: the species, the season within which a census period fell, the experimental site and the age group of the seedling. Seasons in 1989 and 1990 were defined by the calendar months, with autumn defined as March, April and May, winter defined as June, July and August, spring as September, October and November and Summer as December, January and February. Age groups were defined as less than or equal to one month old, between one and three months old, between three and six months old, between six and 12 months old and more than 12 months old. Age group was treated as an ordinal variable. Seedlings arising from different seedlots were agglomerated into the species classes, *E. delegatensis* and *E. amygdalina*.

To examine the impact of time of sowing and time of germination on the average rate of mortality during the course of a cohort's development and the effect of a severe frost event, a regression modelling approach was undertaken. As distinct from the previous analysis, which relied on pooled data to identify if any particular season of the year is more hazardous than others to particular age classes, this approach investigates the form of the survival curves of individual cohorts. A negative exponential model, and one modified to allow for a discontinuity, were fitted.

The first model, the negative exponential model (Harper 1967; Hett and Loucks 1968) is given by,

$$y=y_o e^{-bx}, \qquad (1)$$

where y is the number of any age cohort, y_o is the initial input into the population at time zero, b is the mortality parameter and x is the age of the cohort. This model, in theory, presumes that the probability of mortality is constant throughout the lifetime of an individual, which is to say that in any given time interval an equal proportion of seedlings will die. It is important, however, when interpreting the results of the fit to this function to realise that the fit to the negative exponential model is stongly influenced by early rates of mortality. A good fit does not necessarily support the assumption of a constant rate of mortality throughout an individual's life. In fact in the context of the monitoring frequency in this study the mortality rate parameter, b, is probably better interpreted as an index of the mortality risk immediately following emergence. Effectively this allows a more detailed look at the mortality hazard of different

emergence times than does the logistic analysis. Inferences about later age survival, however are better inferred from the logistic analysis.

A second model is to allow for catastrophes which are reflected as a discontinuity in the hazard function. Such events might be particularly severe frosts such as were recorded during May 1990 that caused widespread mortality. This model may be written as:

$$\text{if } t<pt \;\; y=y_0e^{-bx},$$
$$\text{if } t>pt \;\; y=y_0e^{-bx} - cy_{pt}, \qquad (2)$$

where t is the current time, pt is the time of the perturbation, y_{pt} the number of seedlings alive immediately prior to the perturbation and c is the proportion of these killed. Logically, c must be constrained to always be greater than or equal to zero, since by definition a cohort can have no recruitment into it. Equation (1) can be regarded as a specialised case of (2) where there is no perturbation (ie. c=0). Model (2) will always give an equivalent or better fit to the data than model (1), however such improvements involve the loss of degrees of freedom and such changes may not always result in a significant improvement of the fit to the data. Because (1) is a specialised case of (2), analysis of deviance can be used to compare the fit of the models to the data for any one cohort. To do this the most general form of the equation is fitted, in this case equation (2). Then the less general equation is fitted, in this case equation (1). The gain from the general model over the specific model is calculated as the residual sums of square of the general model minus the residual sums of squares from the specific model. This is then divided by the change in degrees in freedom and used in an F test in which the residual mean square under the specific model is used as the denominator (McPherson 1990). If it is evident that a substantial number of the fitted regressions has been improved, the treatment combinations which are showing a significant improvement in fit can be examined for any underlying reason and specifically whether there are certain types of cases in which one model is superior to the other. Having selected the most appropriate model, the estimated mortality parameters for the different treatment groupings can be compared using the type III model of the GLM module of SAS (SAS 1989).

A similar modelling procedure was used to explore growth. The null hypothesis in analysis of height is that seedling size is only affected by the age of the seedling. To explore the questions of how site, seed, and germination time may influence growth, the general allometric growth curve, suitably linearised, relating height to age can be fitted:

$$\log(\text{Height})=B_0+B_1\log(\text{age}) \qquad (3)$$

The slope parameter, B_1, is the allometric growth rate, and can now be examined for main effects and interactions. Seedlings germinating after 13/11/89, with only three height scorings, were excluded because it was felt that seasonal growth rate variations may bias the end result. This because a seedling that germinates in May and is measured in April the next year may appear to be growing more slowly than one that germinates in September, merely because height increment is measured across one winter, during which growth is negligible, and one spring and summer, compared to the spring germinant that has only been assessed over the growing seasons of spring and summer. To provide sufficient replicates within time of germination classes in the analysis of variance, germination times from different times of sowing were pooled.

6.4 Results

6.4.1 Weather

Mean daily temperature and estimates of surface volumetric soil water content are given in Fig. 6.3a for the Mount Connection site and Fig. 6.3b for the Bicheno site. The soil dryness index for each site was calculated on a daily basis using the algorithm of Mount (1972) as is shown in Fig. 6.4.

Comparison against long term meteorological records from the Lake Leake, Bicheno and Swansea meteorological stations shows that maximum and minimum temperatures for 1989 and 1990 were slightly above average. The average monthly maximum temperatures on nine of the 16 months and the minimum temperatures on 11 of the 16 months were more than 0.5°C above average. In only four months and one month respectively were monthly temperature maxima and minima more than 0.5°C below the long term average. These below average monthly maxima occurred in late spring and early summer 1989, and the below average minimum monthly temperature in May 1990. The maximum temperatures recorded by the maximum-minimum thermometers at Mount Connection and Bicheno respectively were 38° and 37°C , and the minimum temperatures -8° and -5°C respectively. Rainfall records for Lake Leake indicate that rainfall in the period March 1989 to June 1990 was 80% of the long term average. With the exception of February 1990, every month between November 1989 and June 1990 had below average rainfall.

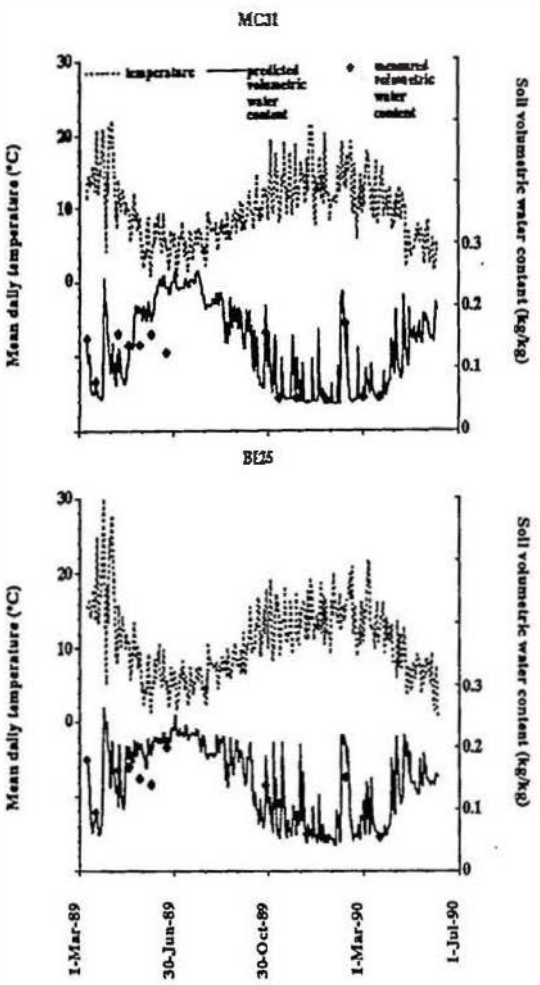


Fig. 6.3. Mean daily air temperature and predicted mean daily soil volumetric water content at both experimental sites.

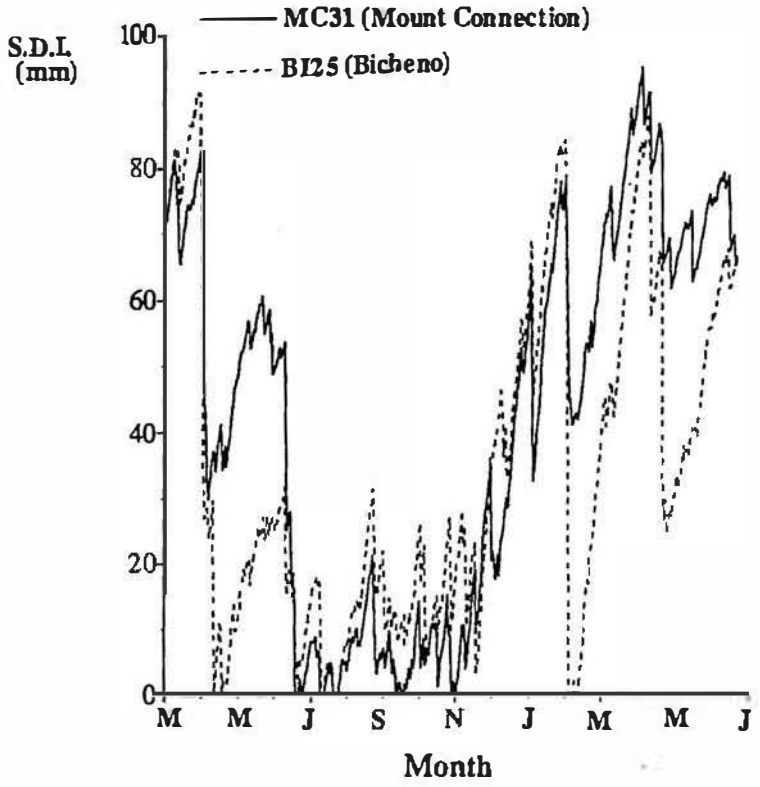


Fig. 6.4. Soil dryness index calculated using the algorithm of Mount (1972) for each experimental site between March 1989 and July 1990.

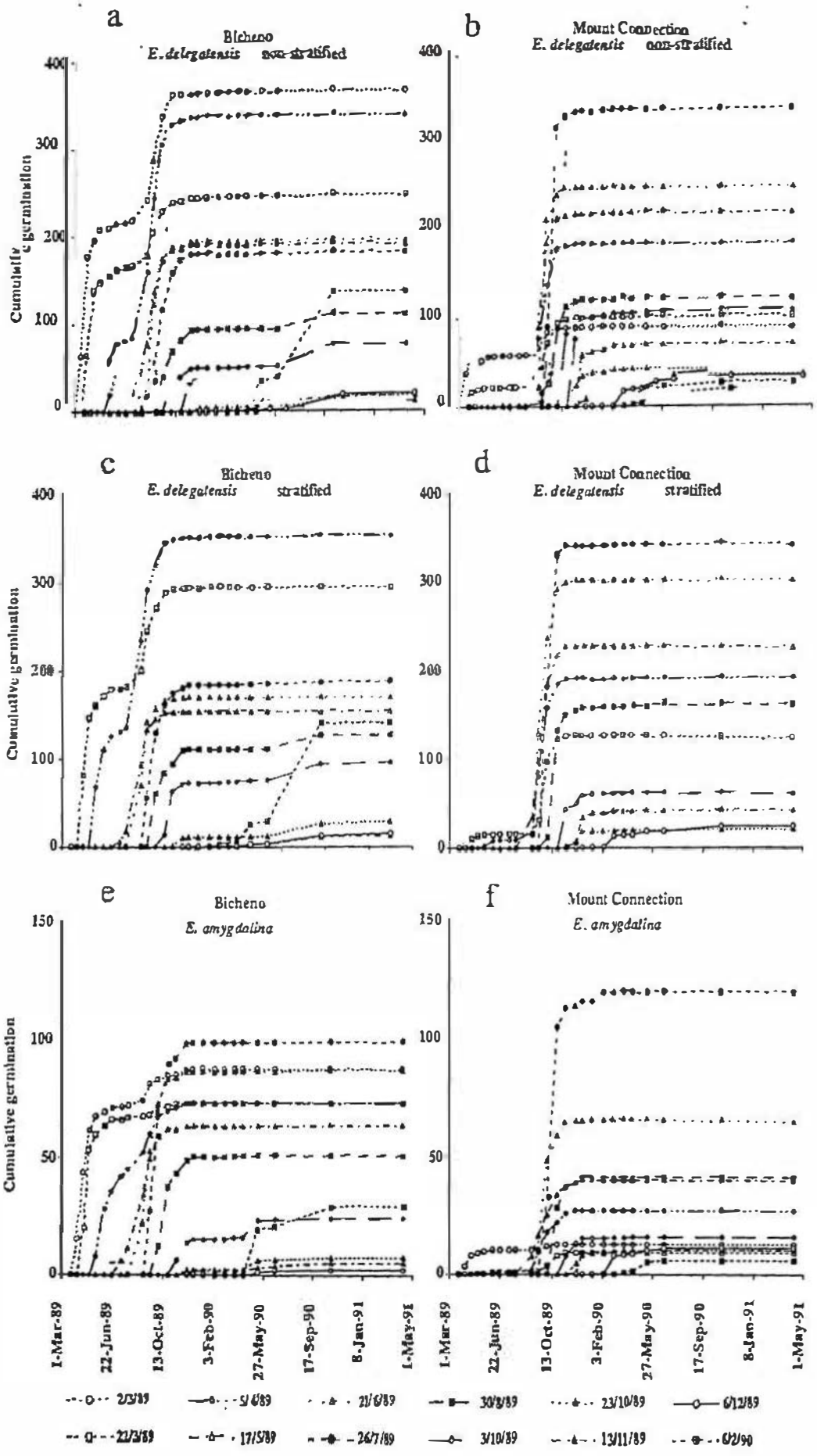


Fig. 6.5. Patterns and timing of cumulative emergence from different times of sowing of the respective seed types at the two experimental sites.

6.4.2 Pattern of germination

Any one time of sowing resulted in significant emergence recorded over six or more scoring periods. Usually, however, at least 80% of total emergence was recorded in three or fewer scoring periods, often reflecting two recruitment episodes, one in the spring and one in the autumn. While some emergence occurred in summer and winter, germination was overwhelmingly concentrated into two pulses, one in spring and one in autumn (Fig. 6.5). Very little seed was observed to germinate from a sowing after the seed had experienced one spring and one autumn, and it seems safe to assume that emergence is effectively complete twelve months after sowing. Emergence from late winter and early spring sowings at both sites was largely compressed into one recruitment episode, of two or three contiguous cohorts, in the spring. Autumn 1989 sowings of *E. delegatensis* resulted in a bimodal distribution of emergence times with flushes in autumn and the subsequent spring. Sowings of *E. delegatensis* made late in the autumn of 1989 at the Mount Connection site resulted in predominantly spring germination. At both sites, early autumn sowings of the non-dormant *E. amygdalina* seed result predominantly in autumn germination, although there was a small proportion of residual germination the following spring (Fig. 6.5e and 6.5f). Later autumn sowings at the Mount Connection site did not result in germination until the following spring. Emergence from seed sown in the summer of 1989-90 at the Bicheno site germinated predominantly in the spring of 1990. Stratification had no clearly discernible effect on the timing or pattern of emergence at either site (compare 6.5a with 6.5c & 6.5b with 6.5d).

These patterns of germination can largely be explained by correlating the occurrence of germination with ambient conditions. Laboratory studies in Chapter 2 indicated that at soil matric potentials below -0.5 MPa (soil water potential for the soils of the texture found at the study sites can be related by the equation: water potential (in J/kg)=-exp(11.27-40.18*soil volumetric water content), with the result that a soil volumetric water content of 0.18 kg/kg corresponds to a soil water potential of -0.5 MPa) , germination is almost entirely inhibited. At temperatures less than 10°C, germination proceeds very slowly but the rate then increases almost linearly up to 20°C, with an average seed requiring approximately 6500 hour degrees for germination. These limits can be used partially to explain the timing of field germination. The commencement of germination in autumn 1989 at both sites corresponded with the wetting of soils to above -0.5 MPa in early April (see Fig. 6.3). Opportunities for autumn germination at the Mount Connection site were, however, truncated by a

subsequent dry spell. By contrast, soil matric potentials at Bicheno site dropped only briefly below the threshold value. When soils at Mount Connection were again sufficiently moist for germination in May, temperatures were above 10°C for only short periods on a few days interspersed by periods of a week or more when conditions were too cool for germination to progress. By contrast, conditions at Bicheno remained sufficiently warm and wet for germination until the end of May. Prolific germination recommenced at the Bicheno site in early September following a rise in temperature during late August. Conditions were slightly cooler during late August and early September at Mount Connection and this may explain the two week delay in the spring flush. As soils dried at both sites during November germination decreased, although short wet periods during summer seemed to be adequate to allow sporadic germination. Autumn 1990 was dry, and consequently conditions suitable for germination in autumn 1990 were short in comparison to 1989 and very little germination was observed. Vigorous germination of seed sown in late spring and summer of 1989/90, where it did occur, was delayed until spring 1990.

6.4.3 Cumulative Emergence

The date seed was sown dramatically influenced the proportion of seed observed to germinate ($P<0.001$). The pattern of response at the two sites was markedly different ($P<0.001$) (Fig. 6.6a, Table 6.2a). There was a distinct site dependant optimum sowing time, from which about 25-30% (40% being the maximum for *E. delegatensis*) of the estimated viable seeds sown were observed as seedlings. At the Bicheno site, early to mid-autumn sowings resulted in the highest cumulative germination, with a gradual decline in observed emergence the later in the year seed was sown. By contrast, winter through to early spring (the sowing made on 30/8 is interpreted as representing early spring) sowings were best at the Mount Connection site. At both sites sowings made in late spring and early summer were particularly unsuccessful, with only a few per cent of the viable seeds sown detected as seedlings. Both species, stratified or non-stratified, responded similarly to different sowing times ($P>0.05$).

The number of seedlings surviving at the end of the experiment at each site showed a similar pattern of response to sowing time as did cumulative germination (Fig. 6.6b, Table 6.2b). At the Bicheno site the average mortality rate was far lower and this "echoing" is far more distinct. At Mount Connection where survival was poor, the net difference between sowing times was low at the end of the experiment but, nevertheless, the original pattern of response was

partially retained. At both sites establishment of *E. amygdalina* from autumn sowings was less successful than was the establishment of *E. delegatensis*, but establishment from winter and spring sowings, for which germination was concentrated in spring, was more successful (Fig. 6.6c). This is reflected in a significant interaction between seed type and time of sowing (Table 6.2b).

Table 6.2. Effect of time of sowing on cumulative emergence and the number of seedlings surviving at the end of the experiment.

a. Cumulative emergence.

Source	*DF*	*Sum of Squares*	*Mean Square*	*F value*	*p*
SITE	1	3.2909	3.2909	14.32	0.0003
SEED	2	0.5894	0.2947	1.28	0.2822
TOS*SEED	21	7.2560	0.3455	1.50	0.0955
SITE*SEED	2	1.3676	0.6838	2.98	0.0559
SITE*TOS*SEED	21	7.0548	0.3359	1.46	0.1113
ERROR	92	21.1663	0.2301		
*Tests of hypotheses using the MS for SITE*TOS(REP) as an error term*					
TIME OF SOWING (TOS)	11	190.2034	17.2912	27.87	0.0001
SITE*TOS	11	43.7939	3.9813	6.42	0.0001
ERROR	48	29.7785	0.6204		

b. Seedlings surviving at 29/10/90.

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*p*
SITE	1	251.2816	251.2816	428.48	0.0001
SEED	2	0.5684	0.2842	0.48	0.6175
TOS*SEED	21	25.9368	1.2351	2.11	0.0082
SITE*SEED	2	0.5330	0.2665	0.45	0.6362
SITE*TOS*SEED	21	14.7339	0.7016	1.20	0.2733
ERROR	92	53.7893	0.5847		
*Tests of Hypotheses using the MS for SITE*TOS(REP) as an error term*					
TIME OF SOWING (TOS)	11	108.0008	9.8182	7.93	0.0001
SITE*TOS	11	71.4764	6.4978	5.25	0.0001
ERROR	48	59.3957	1.2374		

Table 6.3: Effect of timing of seedbed preparation on number of seedlings (Non-stratified *E. delegatensis* seed) recorded at the Bicheno site on the 4/90 from different sowing times. Statistically similar groups are identified using Tukey's multiple comparison method (p=0.05).

Time of sowing	Seedbed prepared at commencement of experiment only		Seedbed prepared immediately prior to each sowing time	
	No. Seedlings	Homogeneous Means	No. Seedlings	Homogeneous Means
2/3/89	220.3	A	257.0	A
22/3/89	151.7	B	233.7	A
19/4/89	110.0	BC	146.7	AB
17/5/89	87.0	BCD	140.7	AB
21/6/89	52.7	CDE	140.3	AB
26/7/89	43.3	DE	132.0	AB
30/8/89	29.7	DE	125.0	AB
3/10/89	12.3	E	72.3	B
23/10/89	8.7	E	61.7	B
13/11/89	3.3	E	18.3	B
17/12/89	0.3	E	17.0	B
16/2/90	0	E	9.7	B

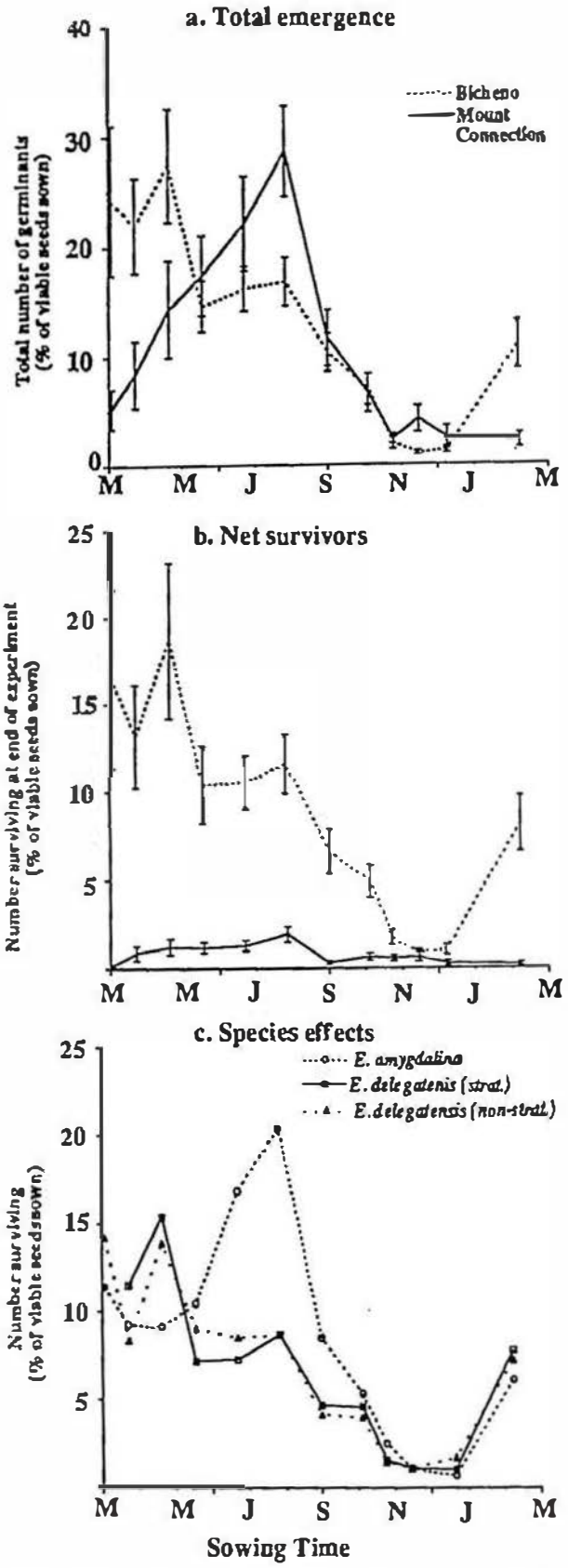


Fig. 6.6. Site and species effects on total emergence and net survival following different sowing times: a. Site effects on total emergence; b. Site effects on net survival; c. Species effects on net survival.

6.4.4 Effect of elapsed time since seedbed preparation

At the Mount Connection site no difference was observed between the number of seedlings surviving on different seedbeds ($P>0.1$). The effect of seedbed differences on total emergence however may well have been masked by the very high mortality rate. At the Bicheno site the mortality rate was low, and the number of seedlings surviving on 29/10/90, when germination was complete on all plots, is an accurate indication of the number that actually germinated. These data may be used to examine the effects of elapsed time since seedbed preparation on cumulative emergence. Unfortunately, in this study the time elapsed since seedbed preparation is confounded by seasonal influences. This has two potential effects. There is a seasonal influence on the loss of seedbed receptivity because of different rates of recolonisation by competing species, and to a lesser extent because of rate differences in the loss of favourable microsites due to factors such as soil crusting and rain wash. Secondly, the importance of seedbed conditions on germination and establishment may vary with season and hence the time of sowing (see Chapter 4). In practice, seedbeds will generally be prepared in late summer or autumn and a given delay will generally cover the same part of the year. It is only in the comparatively rare cases of coupes prepared in spring and held over for autumn sowing that the effect of a given time delay is likely to be subject to different seasonal influences.

The absolute difference between seedling numbers on new and old seedbeds was affected by the proportion of seed that emerged (Fig. 6.7). When emergence was low, the absolute difference between seedbed preparation times, although significant ($P<0.05$), was slight. Importantly, however, for the least successful time of sowing, 13/11/89, no seedlings were observed to be surviving on the seedbed prepared at the beginning of the experiment only (the ratio is obtained by adding 1 to both the numerator and the denominator of the ratio), whereas a few seedlings, approximately one per cent of the viable seed sown, were detected on the seedbed prepared immediately prior to sowing.

A significant relationship ($P<0.01$) exists between the logarithm of the ratio of the seedling numbers on new and old seedbeds and the time elapsed since seedbed preparation ($\log(\text{ratio})=0.120+0.050*(\text{weeks elapsed})$, $r^2=0.59$), but this should be interpreted judiciously because of the very low final numbers of seedlings from some times of sowing (Fig. 6.7). The logarithmic relationship indicates a highly significant multiplicative effect of elapsed time. As would be expected from the hypothesis that the difference is due to time since seedbed

preparation the intercept is not significantly different to zero ($P>0.1$). Care must, however, be taken in interpreting these ratios because of the very low seedling numbers during the winter months, with small absolute differences in numbers resulting in large differences in the ratio of seedling numbers between the treatments.

While seedbed preparation time strongly influenced the number of seedlings observed to germinate, the ranking of time of sowings by total number of observed germinants was generally the same. However, on seedbeds prepared immediately prior to sowing there was less distinction between sowing times, with only two statistically different groups being determined using Tukey's test method for multiple comparison, whereas for seedbeds prepared at the commencement of the experiment only, the times of sowing fell into five significantly different groups (Table 6.3). Because the most favourable sowing times were those made earliest it appears that time since seedbed preparation acted to accentuate the difference between the most favourable and the least favourable sowing times.

6.4.5 Seasonal variation in seed harvesting

The pattern of seed removal displayed a distinct seasonal trend at the Mount Connection site, with a higher proportion of seed, approximately 40%, removed during the warmer months of the year and virtually no seed removed in the coldest months of the year. At the Bicheno site, while seed harvesting was low in winter relative to times of very high harvesting intensity in autumn, approximately 30% compared with 85%, a moderate level of browsing was observed in all months of the year (Fig. 6.8). The differences between sites and times of sowing were found to be highly significant, whereas the interaction was not (Table 6.4). At both locations some bait sites were consistently harvested heavily, some only harvested during the warmer months of the year, and some were never harvested. This would suggest that some were positioned closer to concentrations of seed foragers and that activity was seasonally influenced.

Table 6.4. Repeated measures analysis of variance for site and seasonal variation in seed browsing.

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*p*
Between subjects stratum					
SITE	1	1161.6	1161.6	7.66	0.0127
ERROR	18	2729.1	151.6		
Between times within subjects stratum					
TIME OF SOWING (TOS)	11	894.7	81.33	3.72	0.0001
TOS*SITE	11	299.3	27.21	1.24	0.2601
ERROR	198	43303.3	21.87		

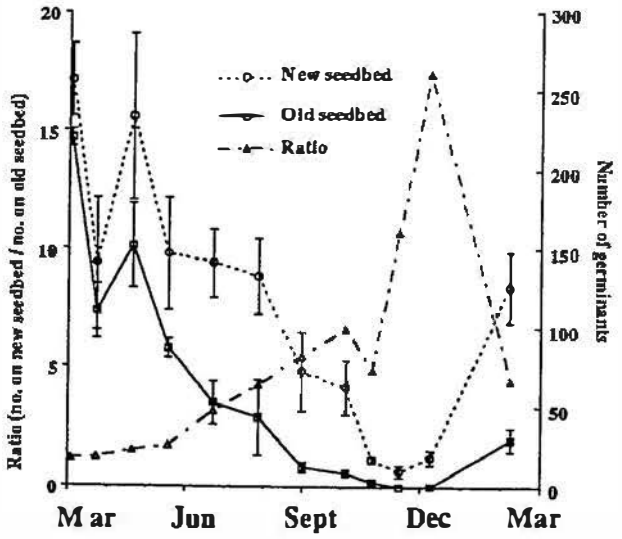


Fig. 6.7. The effect of time elapsed since seedbed preparation on the number of seedlings surviving at the Bicheno site on 11/4/91. "New seedbeds" were prepared immediately prior to sowing, "Old seedbeds" were prepared on the 26/2/89 only.

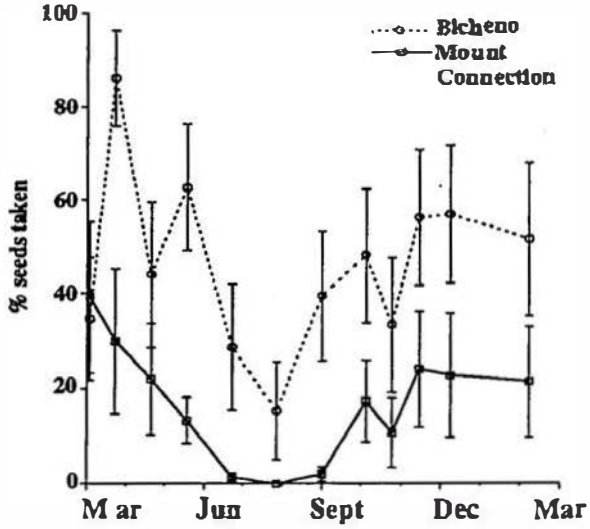


Fig. 6.8. Seasonal variation in the intensity of seed harvesting at the two experimental sites. Error bars are the 95% confidence interval of the mean.

6.4.6 Survival

Survival of 1200 identified cohorts (time of sowing by time of germination by site by seed combinations) was followed. However, some of these cohorts had very few individuals, and were unsuitable for modelling purposes. Only 432 of these cohorts, each with more than 10 individuals, and for which survivorship had been recorded for more than three time periods, were included in the subsequent analysis. Of these, 264 were from the Bicheno site and 168 from the Mount Connection site. A selection of mortality curves for different combinations of time of sowing, site and seed type are presented in Fig. 6.9 to indicate the range of responses. A full listing of all the survival curves is given in Appendix 3. The contribution of different times of germination to the total population can be seen, in particular the contribution of autumn and spring germinants from autumn times of sowing (Fig 6.9d). From the curves it appears that spring germinants from autumn and spring sowing times follow similar trajectories. It is immediately obvious that mortality at the Bicheno site has been substantially less than at the Mount Connection site (Fig. 6.9d and 6.9e). The impact of a severe frost on mortality at the Mount Connection site during May 1990 is visible (Fig. 6.9d&e). These observations can be more formally testing using the modelling approach already introduced. Specific questions that arise are: 1) is the mortality hazard age-related?, 2) are certain times of germination more hazardous and does the time of sowing have an influence on this?, 3) is the frost event at the Mount Connection site best accounted for by introducing a discontinuity into the survival curves?

Logistic analysis indicated that site, age group, species and season all contributed significantly to the probability of mortality between census periods. The highly significant Chi-Square statistic of the residual, however, indicates a significant amount of unexplained variation (Table 6.5). The rate of mortality was significantly higher at the Mount Connection site than at the Bicheno site. Generally younger age groups were more likely to die ($P<0.001$: Fig. 6.10). This trend was most accentuated for *E. amygdalina* seedlings which were more susceptible to mortality in young age groups than were seedlings of *E. delegatensis* ($P<0.05$). Because mortality was recorded as occurring at the end of a census period, care needs to be used in interpreting seasonality effects and this is most evident for the frost event that caused extensive mortality at the Mount Connection site near the end of May 1990. Because the census period ran from 14/5/1990 to 22/6/1990 this was recorded as having occurred in the winter of

1990 (Fig. 6.10). The very high mortality rate associated with the frost of the second autumn is evident in the very high mortality rate of the winter of 1990 at the Mount Connection site. The effects of this event appear to have been relatively independent of age. Mortality of *E. delegatensis* in the second summer at the Mount Connection site also appeared not to follow the general trend of an inverse relationship with age . Many of these individuals, however, were seedlings which, while not killed outright by the frost of the previous winter, had been frost heaved so that a large proportion of their root systems were exposed. The youngest seedlings had already died during the winter and spring but the older seedlings survived until early summer. With the noted exceptions of winter and summer 1990 the pattern and magnitude of seasonal effects were relatively constant between years and seasons. On the basis of the logistic analysis, there appears to be no one time of year that is, on average, more hazardous to a particular age class of seedlings than any other. Although a traditional classification of seasons by months was used in this analysis, this is no guarantee of any correspondence to the temporal distribution of hazard; high risk periods may overlap seasons obscuring relationships. Field observation showed each season to hold different risks, but that some factors overlapped seasons. During the winter months mortality resulted predominantly from frost heave. These deaths were more location dependent than age-dependent. Areas of loose wet soil were particularly susceptible to frost heave. In early spring, fungal attack appeared to be severe. During late spring, summer and early autumn drought and defoliation by grasshoppers and chrysomelid beetles were major causes of death. During late autumn and early winter, insect defoliation was severe and 'dampening off' from fungal attack had once again established as a significant cause of death.

From Figs. 6.9 & 6.10 it is clear that at the Mount Connection site, at least, the strongest evidence of a discontinuity in survivorship curves occurred in May 1990. Fitting model (2), which allows for such a perturbation, over model (1) which assumes a constant probability of death, caused a significant reduction in deviance for 31 of the 168 cohorts from the Mount Connection site, and, not unexpectedly, for none of the cohorts from the Bicheno site. Fitting model (1) led to a mean r^2 at the Bicheno site and Mount Connection site of 0.72 and 0.84, respectively. Fitting model (2) improved the mean r^2 at the Mount Connection site only slightly to 0.87. Examples of good fits of models (1) and (2) to sample data sets are shown in Fig. 6.11. Even in the case of a good fit of model (1) (Fig. 6.11a), the impact of the frost event on the survivorship curve is discernible.

Table 6.5. Analysis of variance table for the interaction of the effect of categorical variables site, species, season and age group on probability of surviving the interval between censuses.

Source	*DF*	*Chi-Square*	*Prob*
Intercept	1	2.69	0.1007
Site	1	10.38	0.0013
Age Group (Age)	1	27.71	<0.0001
Age*Site	1	1.40	0.2375
Season (Seas)	6	30.19	<0.0001
Site*Season	6	141.95	<0.0001
Age*Seas	6	16.13	0.0131
Age*Site*Seas	6	98.17	<0.0001
Species (Spp)	1	6.29	0.0121
Site*Spp	1	3.02	0.0821
Age*Spp	1	4.82	0.0281
Age*Site*Spp	1	3.04	0.0812
Seas*Spp	6	10.65	0.0999
Site*Seas*Spp	4	5.23	0.2644
Age*Seas*Spp	6	15.06	0.0198
Age*Site*Seas*Spp	4	3.28	0.5127
Residual	38	990.75	<0.0001

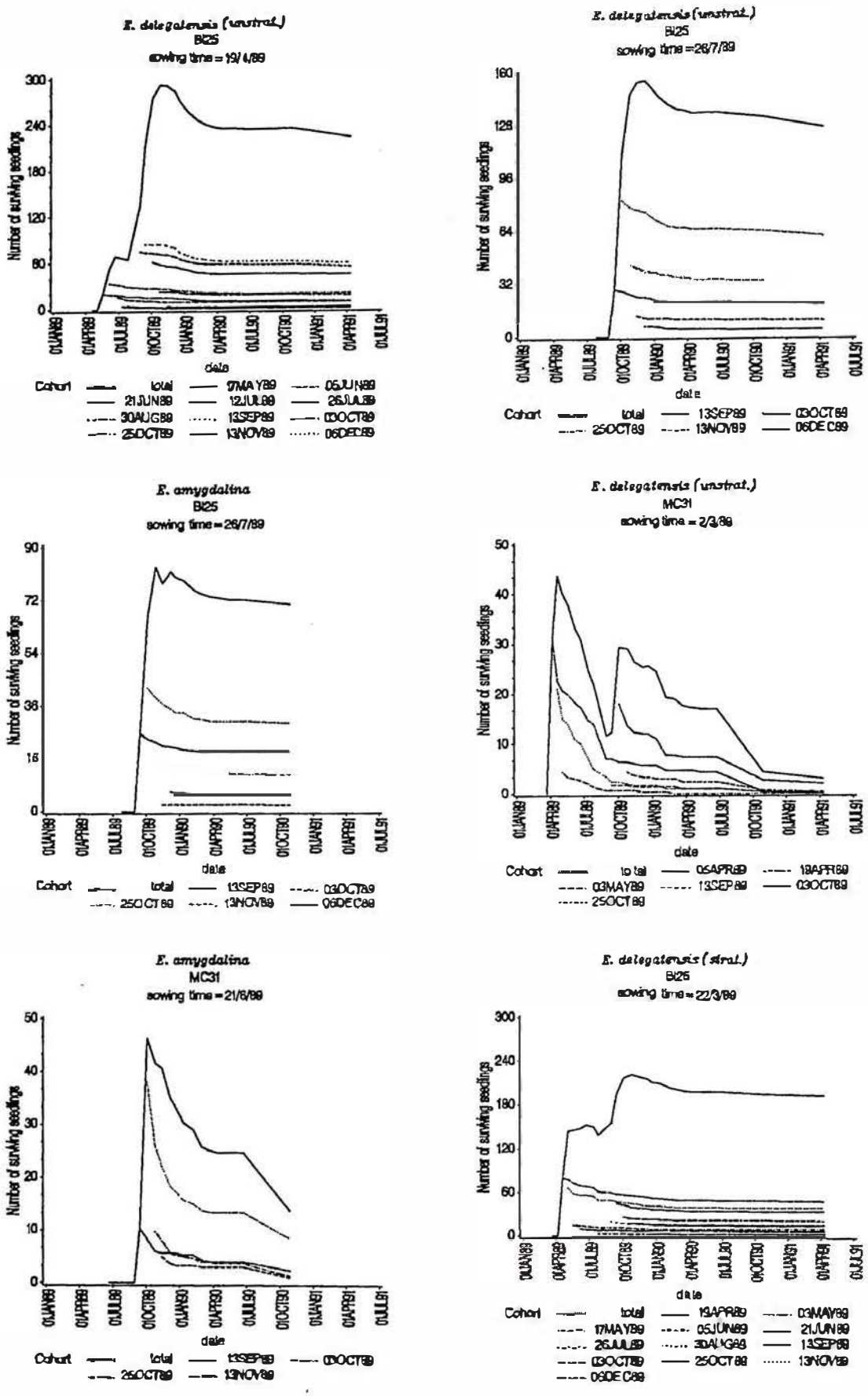


Fig. 6.9. Examples of survivorhip curves for some of the 1200 cohorts studied. Each graph represents the mean response of three replicates.

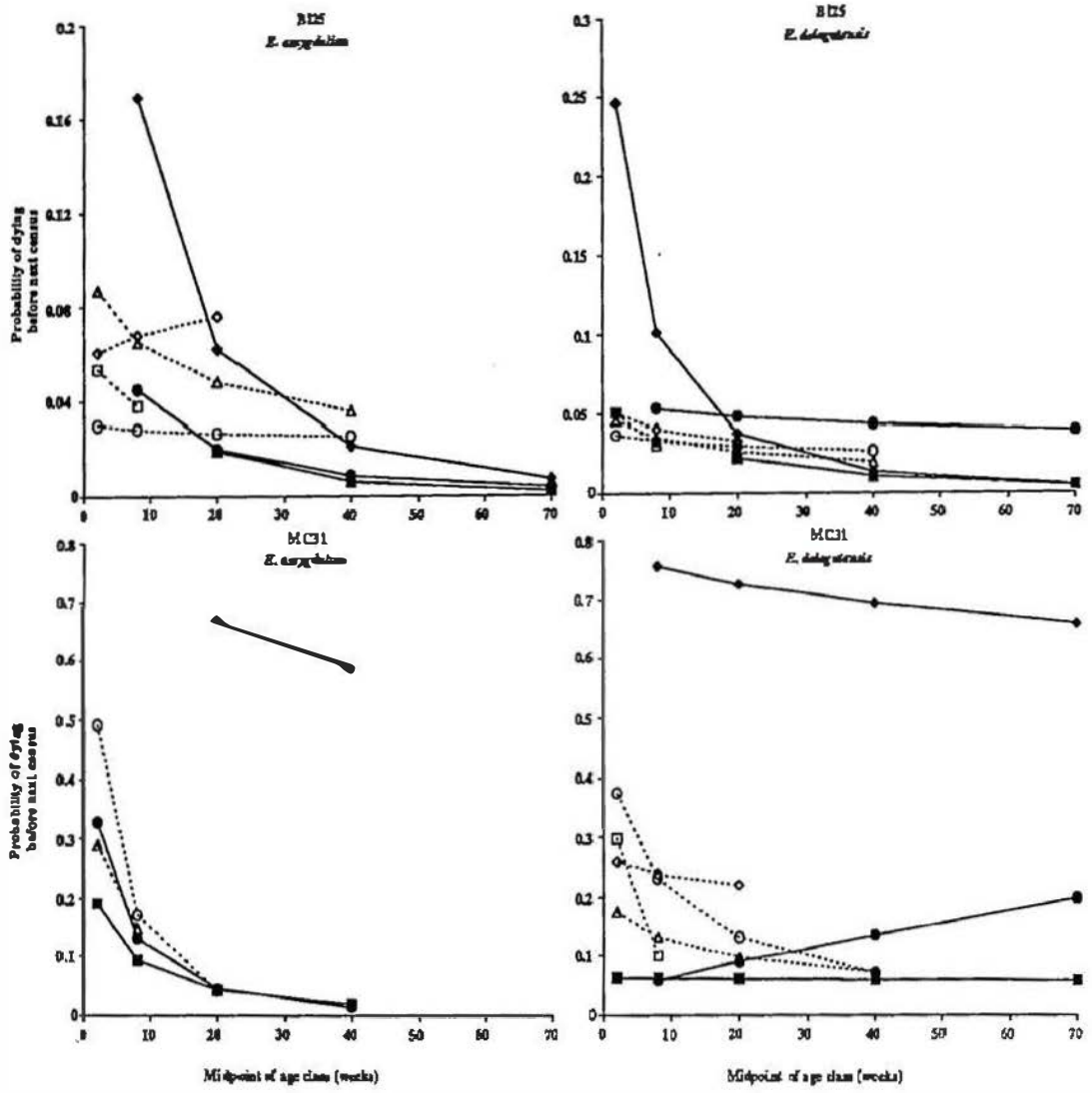


Fig. 6.10. Seasonal variation in the probability of dying between censuses. Hollow symbols represent the seasons of 1989, filled symbols 1990. □ Autumn ◇ Winter △ Spring ○ Summer.

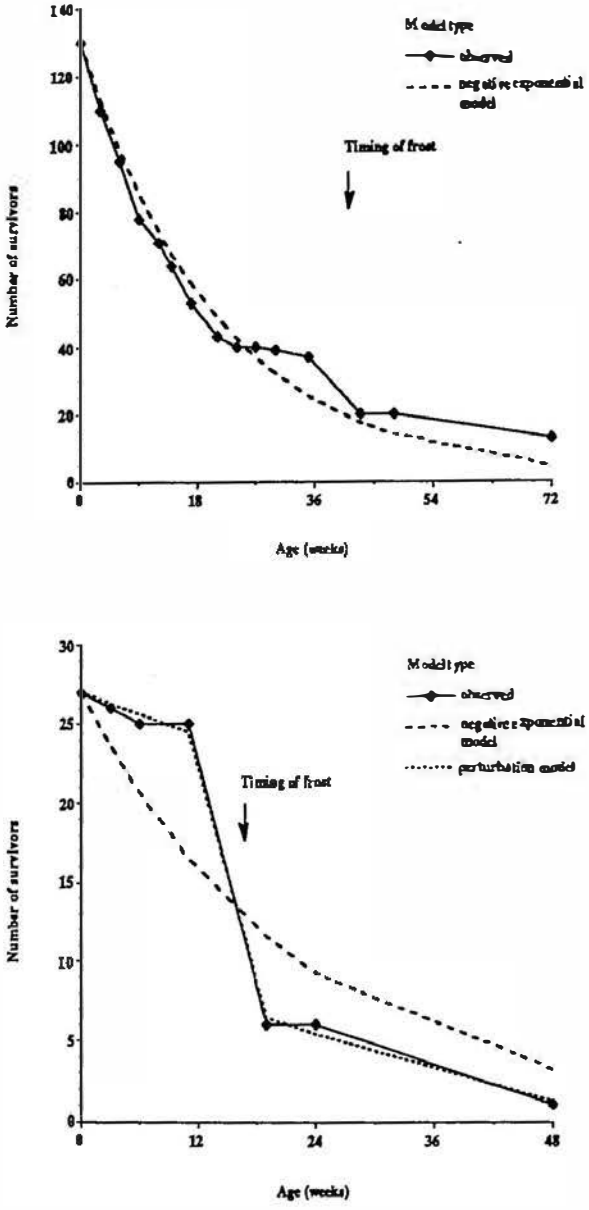


Fig. 6.11. Example of a data set for which the negative exponential model provided a satisfactory fit and one for which a discontinuity in the survival curve to account for a frost event was required.

The parameters from model (2), b and c, represent the mortality rate and the probability of being killed by the frost event of May 1990. The average rate of mortality was strongly influenced by site and, contrary to what might be expected from the logistic analysis, the time of germination (Table 6.6a). As seen in the previous analysis, mortality was significantly higher at the Mount Connection site (b=0.0764; cf 0.0104 at the Bicheno site), the estimated time for 50% of seedlings to die being 63 days compared with 466 days at the Bicheno site. There was no indication that any times of germination were significantly more hazardous than any other at the Bicheno site. At the Mount Connection site, however, late autumn through to early winter and late spring through early summer were clearly hazardous emergence times relative to the comparatively safe emergence times of early autumn and early spring (Fig. 6.12). These hazardous times overlap seasons, late autumn/early winter and late spring/early summer, and this is perhaps why the division into traditional seasons in the logistic analysis failed to highlight consistent within year times of high hazard.

Times of sowing resulting in emergence concentrated at these hazardoustimes gave rise to higher average mortality rates (Fig. 6.13). Mid to late spring times of sowing were, therefore, particularly poor. Mid to late autumn times of sowing, with germination split between early winter and early spring, were intermediate in performance. Mid to late winter times of sowing resulted in outstanding seedling survival rates with early autumn times being the next most consistently favourable time. Survival of autumn germinants from autumn sowings was generally poorer at both sites than was the survival of spring germinants from autumn sowings. At the Mount Connection site early spring germinants from late winter and spring sowings had a longer half life than did late emergents, but this effect was not manifest to the same extent at the Bicheno site (Fig. 6.14). By comparison, the effect of seed type was slight. At the Bicheno site the mean half life of *E. amygdalina* seedlings was significantly less than *E. delegatensis* (336 days compared with 515 days). By contrast, at the Mount Connection site mean half life was greatest for *E. amygdalina* (80 days compared with 72 and 53 days respectively for stratified and non-stratified *E. delegatensis* seed).

In addition to the obvious effect of site (P<0.001) on the proportion of seedlings killed by the frost in May 1992, represented by c in model 2, several higher order interactions were observed using analysis of variance (Table 6.6b). The higher order interactions gave rise to consistent pattern, and in general terms accelerated mortality due to the frost event appears to have been relatively independent of time of germination or time of sowing.

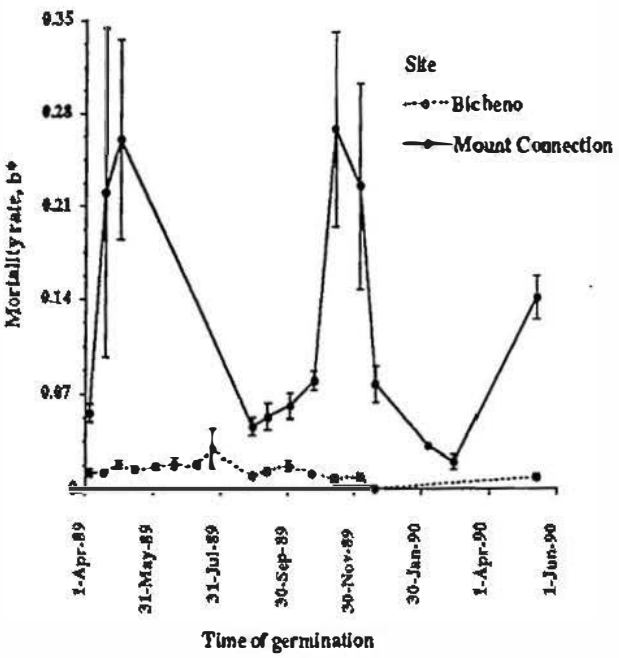


Fig. 6.12. The interaction of site and time of germination on the rate of mortality. Error bars are the 95% confidence interval of the mean. * parameter from equation (2).

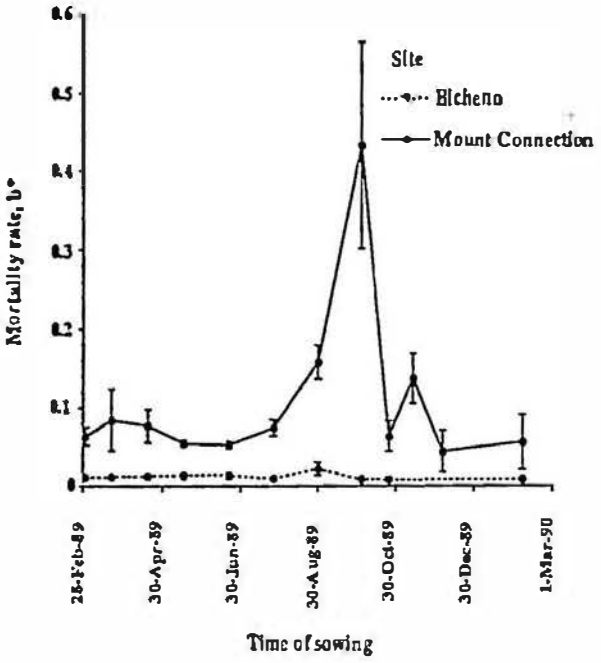


Fig. 6.13. The interaction of site and time of sowing on the rate of mortality. Error bars are the 95% confidence interval of the mean. * parameter from equation (2)

Table 6.6 Analysis of variance of the parameters from the mortality model: survivors = number to germinate*e(-*bage)-*c**(survivors as of 14 May 1990);**

a. The mortality rate, *b*.

Source	*DF*	*Sums of Squares*	*Mean Square*	*F Value*	*p*
Site	1	1.3196	1.3196	468.65	0.0001
Time of Germination (TOG)	16	0.5079	0.0317	11.27	0.0001
Site*TOG	7	0.2102	0.0300	10.66	0.0001
Site*TOS*TOG	12	0.1257	0.0105	3.72	0.0001
TOS*TOG	29	0.2483	0.0086	3.04	0.0001
Seed*TOG	22	0.0249	0.0011	0.40	0.9920
Site*TOS*Seed*TOG	37	0.0549	0.0015	0.53	0.9863
Seed	2	0.0267	0.0134	4.75	0.0104
TOS*Seed	14	0.0204	0.0014	0.52	0.9191
Site*Seed	2	0.0384	0.0192	6.84	0.0016
Site*TOS*Seed	12	0.0451	0.0037	1.34	0.2084
Site*Seed*TOG	8	0.0221	0.0027	0.98	0.4535
Error	113	0.3181	0.0028		
*Tests using Site*TOS(REP) as an error term*					
Time of Sowing (TOS)	10	0.2441	0.0244	2.27	0.0316
Site*TOS	8	0.1394	0.0174	1.62	0.1477
Error	41	0.4400	0.0107		

b. Added proportion killed by the frost of May 1990.

Source	*DF*	*Sums of Squares*	*Mean Square*	*F Value*	*p*
Site	1	0.9629	0.9629	71.70	0.0001
Time of Germination (TOG)	16	0.1215	0.0076	0.57	0.9036
Site*TOG	7	0.1057	0.0151	1.12	0.3531
Site*TOS*TOG	12	0.4678	0.0390	2.90	0.0016
TOS*TOG	29	0.6508	0.0224	1.67	0.0301
Seed*TOG	22	0.1157	0.0052	0.39	0.9932
Site*TOS*Seed*TOG	38	0.9935	0.0261	1.95	0.0038
Seed	2	0.1550	0.0775	5.77	0.0041
TOS*Seed	14	0.4346	0.0310	2.31	0.0078
Site*Seed	2	0.1824	0.0912	6.79	0.0016
Site*TOS*Seed	12	0.3332	0.0278	2.07	0.0246
Site*Seed*TOG	8	0.0665	0.0083	0.62	0.7604
Error	113	1.5176	0.0134		
*Tests using Site*TOS(REP) as an error term*					
Time of Sowing (TOS)	10	0.4597	0.0460	0.76	0.6634
Site*TOS	8	0.1772	0.0215	0.36	0.9370
Error	41	2.4736	0.0603		

6.4.7 Growth

For all factor combinations growth was slow for the duration of the experiment. Although tallest seedlings were over 20 cm tall at the time of the last scoring, the majority were still below 10 cm tall. Variation in growth rates among seedlings of the same cohort was high. The results of analysis of variance of the allometric growth rate, the parameter B_1 from equation (3), is given in Table 6.7. The units of B_1 are log(cm)/log(day). Seedlings from stratified *E. delegatensis* seed and *E. amygdalina* seedlings grew at the same rate, whereas seedlings from *E. delegatensis* seed that had not been stratified grew slightly more rapidly (non-stratified *E. delegatensis*=0.65 log(cm)/log(day), stratified *E. delegatensis*=0.47, *E. amygdalina*=0.41). Seedlings germinating in the autumn or spring at the Bicheno site grew well relative to other emergence times but, statistically, differences between germination times were not significant ($P<0.1$). At the Mount Connection site, early- to mid-spring germinants grew most rapidly ($P<0.05$) and autumn and the latest of the spring germinants the most poorly (Fig. 6.15).

Table 6.7. Analysis of variance table for growth rate, the rate co-efficient from the allometric growth curve.

Source	*DF*	*Sums of Squares*	*Mean Square*	*F Value*	*p*
Site	1	0.0027	0.0027	0.01	0.9073
Seed	2	1.3888	0.6944	3.45	0.0345
Site* Seed	2	0.3449	0.1724	0.86	0.4267
Time of Germination (TOG)	12	4.7856	0.3988	1.98	0.0302
Site*TOG	7	3.4254	0.0489	2.43	0.0222
Seed*TOG	22	3.5100	0.1595	0.79	0.7302
Site* Seed*TOG	11	1.7767	0.1615	0.80	0.6373

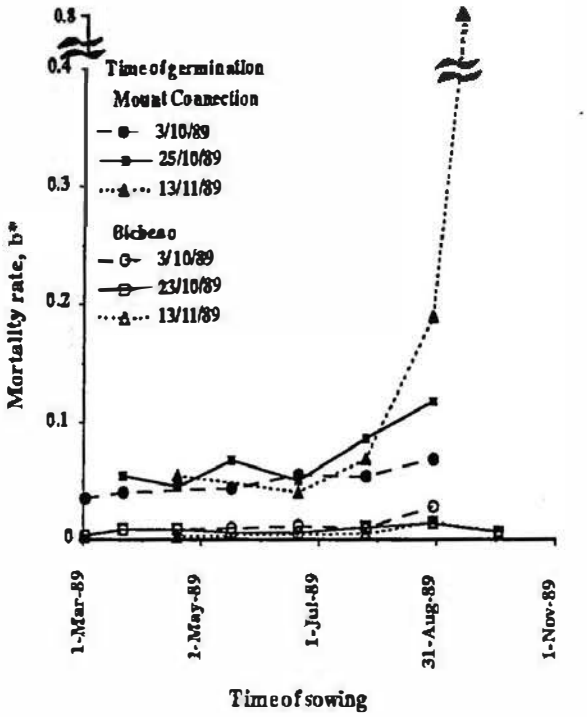


Fig. 6.14. The interaction of time of sowing and time of germination on the mortality rate. Each curve represents like-aged seedlings from different times of sowing.

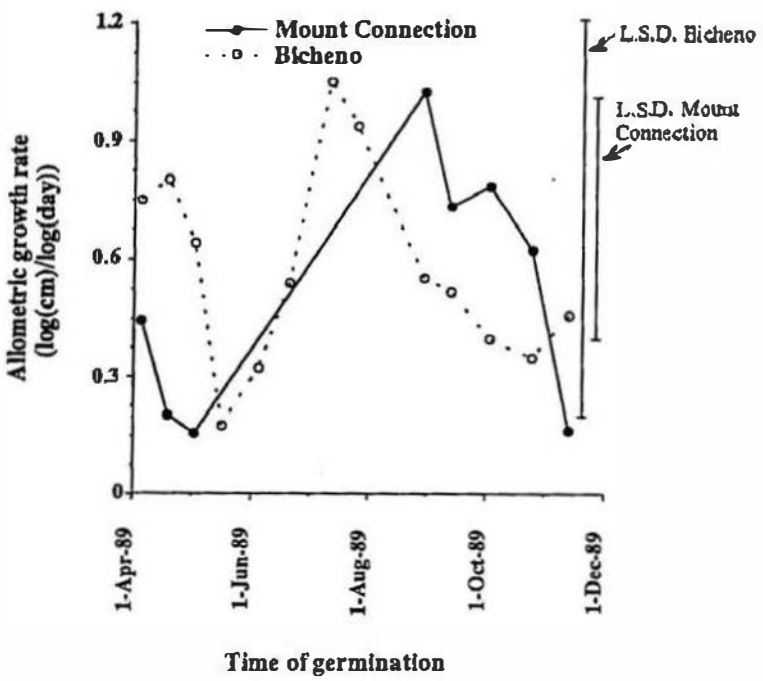


Fig. 6.15. The effect of time of germination on subsequent growth rate. The error bars are the 95% confidence level Tukey-Kramer least significant differences for multiple comparisons.

6.5 Discussion

6.5.1 Germination

Although the two experimental sites used in this study were within 30 km of each other, and did not vary substantially in mean climatic conditions, different times of sowing gave markedly different regeneration outcomes. At both sites, however, similar threshold values of soil moisture and ambient temperature appeared to control the timing of germination flushes. Controlled environmental studies described in Chapter 2 predicted that vigorous germination requires the coincidence of soil moisture levels greater than -0.5 MPa and ambient temperatures greater than 10°C for at least part of the day. These limits appear to describe field processes adequately. In 1989, soil became wet enough for germination to commence at the end of March. Temperatures at the Mount Connection site, however, cooled rapidly as autumn progressed and for only a short period was the temperature warm enough for germination. At the Bicheno site where mean daily temperatures were on average 1.5°C warmer, and where conditions remained warm until late in Autumn, germination continued into early winter. By contrast opportunities for germination in the autumn of 1990 at both sites were slight because of the persistence of dry conditions into May, and consequently germination of late spring and summer sown seed, where it occurred, was held over until spring 1990. The temperature at the Mount Connection site did not become warm enough for germination in spring 1989 until two weeks later than at the Bicheno site, with a consequent delay in the spring germination flush. Suitable conditions for germination then persisted at both sites for almost three months.

The comparative success of different sowing times at the experimental sites can be understood in this context. Autumn sowings at Mount Connection were unsuccessful. Clearly, this can be related to the limited opportunities for germination in 1989, and germination being delayed until the subsequent spring. Many of these seeds stored in the soil for six or seven months were undoubtedly lost as a result of seed harvesting, fungal activity and burial. By contrast, spring-sown seed germinated within weeks of being sown and 40% of the estimated viable seeds were detected as seedlings. Autumn-sown seed at the Bicheno site germinated rapidly and while a portion of the seed fraction over-wintered and germinated the following spring this was presumably only the dormant fraction of seed. This second peak wasn't manifest in the non-dormant *E. amygdalina*

seedlot, but suprisingly was apparent in the *E. delegatensis* seedlot that had been stratified for four weeks, a period identified in laboratory work as sufficient to render 90% of seed non-dormant. It is possible that the partial drying of the seed to allow sowing, or environmental effects immediately following sowing, have negated any advantages of stratification. Both of these seem unlikely. Experiments in which seed has been air dried following stratification (Grose 1963, FCT unpublished data) and the general resistance of Tasmanian seed to the induction of secondary dormancy observed in laboratory studies described in Chapter 2, indicate that changes in the dormancy attributes of seed are unlikely. Nevertheless, the most likely explanation seems to be that the higher number of germinants observed from autumn sowings than from spring sowings at the Bicheno site is due to the additional germination of the dormant seed fraction following over-wintering, whereas this component of the spring sown seed did not survive to germinate the next winter. Similar to this study Grose (1957a), albeit dealing with a seedlot with a greater degree of inherent dormancy and one possibly more susceptible to the induction of secondary dormancy, found that dormant seed sown in spring was destroyed or rendered non-viable by the time stratifying conditions and a subsequent period suitable for germination had occurred.

Very little germination was detected more than 12 months after sowing. Although there have been anecdotal reports of germination delayed substantially longer than this (Lockett 1991), the overwhelming evidence from research is that very little germination occurs after 12-18 months (Grose 1957a; Cunningham 1960; Purdie 1977; Cremer *et al.* 1978; Fagg 1981; Campbell and Bray 1987). Clearly there is no appreciable ground store of eucalypt seed, and unless there is a residual seed source following logging, no reliance can be placed on further recruitment more than one autumn and one spring after sowing. Remedial treatment of an under-stocked area is more difficult the longer the time elapsed since seedbed preparation. Consequently, clearfelled areas should be checked one year after sowing and if an area is found to be significantly understocked remedial action should be undertaken immediately.

While the regeneration niche was defined in part by temperature and soil moisture, the time since seedbed preparation was also an important factor in determining the outcome of any sowing. The ratio of the number of seeds observed to germinate on freshly-prepared, compared with old seedbeds increased with the time elapsed between the two preparation times. The rate of decline in seedbed suitability was slow during the winter months, but in the subsequent

spring weed growth was more vigorous and the decline more marked. Improved germination following sowing soon after seedbed preparation is due in part to reduced weed competition (Cremer and Mount 1965; Campbell and Bray 1987) and partly because sowing seed promptly after seedbed preparation also allows seeds to penetrate into minor crevices and became either partly or totally buried. Eucalypt seed germinates better if it has a shallow covering of loose soil (Cremer 1965), probably largely because this ensures good seed-soil contact which is critical for water uptake and water retention (McWilliams and Phillips 1971; Sheldon 1974). Following the preparation of a seedbed, soil surface heterogeneity is at a maximum, and the work in Chapter 4 has shown that this microtopographical variation is important for seed survival and germination under conditions of fluctuating soil moisture. The longer the delay the more prone soil is to factors such as soil crusting which have been shown to be a major impediment to germination and establishment (Sheldon 1974). In this study, seedbeds prepared in autumn were still acceptable the following spring. However, it should be remembered that both study sites were comparatively dry and not subject to vigorous weed invasion and experience from pastoral situations where weed invasion is more rapid (e.g. Sharp 1985; Weatherly 1985; Oates and Clarke 1987) indicate that a substantial loss of seedbed receptivity during winter is possible. Seedbeds prepared in the spring are unlikely to be prepared early enough for spring sowing. Weed invasion during the spring, and soil crusting during the summer, may impede severely autumn regeneration on such sites.

Although the time of seedbed preparation had a highly significant influence on sowing time outcome, ranking sowing times by the number of germinants gave nearly the same order irrespective of the timing of seedbed preparation. The confounding effect of elapsed time since seedbed preparation, however, increased the sensitivity of germination to sowing time. That is, recently prepared seedbed reduced the effect of time of sowing on the germination outcome. This may well reflect the changing significance of microsites with environmental severity. During adverse germination times a seedbed with many favourable microsites allowed seeds to survive whereas on old seedbeds seeds were killed. Inferences from previous time of sowing work that did not make this distinction may be generally valid, but care should be taken in the interpretation and application of the results because of the possibility of an between interaction the suitability of conditions for germination at the time of sowing and the time elapsed since seedbed preparation.

Seed harvesting was only briefly examined in this work. The findings, however, suggest that seed harvesting may remove a significant proportion of seeds sown. This has been found in a number of other studies examining the fate of eucalypt seed following sowing or natural seedfall (e.g. Pryor and Clarke 1964; Cremer 1966*a*; Purdie 1977; Ashton 1979). The intensity of seed harvesting was significantly different at the two sites examined but at both sites the rate of seed removal followed a similar seasonal trend. Rates of seed removal during the summer and early autumn months were between two and four times higher than during winter months. This may be a result of seasonal variation in temperature which has been emphasized as a major factor influencing the rate of removal of seeds in other studies (Johns and Greenup 1976; Ashton 1979; Andersen 1983).

The actual impact on seedling recruitment is less clear since all seeds removed by ants are not necessarily destroyed. In fact, on several occasions clumps of seedlings emerging from apparently buried seed were observed, suggesting that seeds had been transported into sub-surface chambers, but not to a depth sufficient to prevent emergence. At most 40% of the viable seeds sown were detected as seedlings. With between 40 and 70% of seeds at the Bicheno site and up to 40% at the Mount Connection site being removed from traps, it is likely that a significant proportion of the unaccounted for seeds is lost to seed harvesters. Seed sown late in autumn over-winters and emerges in the spring, however seed sown in late spring and summer results in very little germination the following autumn. Even though autumn of 1990 was not especially conducive for germination, emergence was nevertheless lower than expected. While the apparent difference in survival of seed in the soil between the period autumn through to spring, compared with spring through to autumn, is probably due in part to desiccation of seeds irreversibly committed to the germination process (see Chapter 2), the greater activity by seed harvesters over the warmer months may also be partially responsible. Seed baits at some locations at each site were harvested heavily and repeatedly, and others not at all, probably as a result of the position of baits in relation to colonies of seed harvesting animals Nevertheless, there was little relationship between the intensity to which a trap was harvested and the relative performance of nearby sowing plots. Ashton (1979) found that proximity to nests was highly influential in determining seed removal by ants, and that foraging behaviour of a seed collecting ant, *Prolasius pallidus* Clark (Formicinae), was intensive only within 24 cm of the nest and sporadic up to 75 cm. The total area of sowing plots was considerably larger than this, and this may have masked the localised effect of seed harvesters.

6.5.2 Survival and growth

In general, *E. amygdalina* seedlings at a very young age were found to be more vulnerable to mortality factors than were *E. delegatensis* seedlings (Fig. 6.10). At the Bicheno site where the rate of mortality of older seedlings was very low this effect is detectable as a reduced mean life expectancy. At the Mount Connection site where the average rate of mortality was high, this effect was diminished and there was little difference in mean life expectancy. However, although establishment from autumn sowings of *E. amygdalina* was poor relative to the performance of *E. delegatensis*, establishment from late winter and early spring sowings was superior for *E. amygdalina* even though cumulative germination from sowings in both periods was equivalent (Fig. 6.6c). In laboratory studies a closely related species to *E. amygdalina* (*E. pulchella*) has been shown to be more drought tolerant than *E. delegatensis* (Davidson and Reid 1989). It has also been shown to be less frost resistant (Davidson and Reid 1985). However, the seed of *E. amygdalina* is considerably smaller than that of *E. delegatensis* (Boland *et al.* 1980) and the emergent cotyledonary seedlings smaller. Death of seedlings as a result of frost induced tissue damage was less frequent than was death arising from frost heave, and, consequently, the poorer winter survival of *E. amygdalina* found in this study may have resulted from a greater susceptibility to frost heave as a consequence of seedling size rather than differential frost tolerance.

At the Bicheno site, the average mortality rate was low and there was little distinction in success of different emergence times compared to the Mount Connection site. Nevertheless, at both sites winter was a hazardous time for seedlings of all age classes (Fig. 6.10) Seedlings that germinated late autumn and early in winter were very young when exposed to severe frosts and had markedly reduced life expectancies (Fig. 6.12). At the Mount Connection site late spring and early summer emergence times, immediately prior to the drying of soils during summer, also result in reduced life expectancies. The relatively high mortality rate of mid-spring emergents, a most prolific emergence time at the Mount Connection site, seems at first incongruous. At this time germination was prolific, but it was also comparatively independent of location. For example, seeds were observed to germinate on rocks covered with only a thin film of soil. Sites suitable for germination are not always good sites for survival (Potts 1986, see also Chapter 4), and in this case there was considerable mortality during the next dry spell. Suprisingly mid- to late-summer emergence times were comparatively successful, but at these times germination was largely confined to

depressions (see Chapter 4) where subsequent seedlings may have been buffered from soil desiccation. The combined effects of the two high risk periods, winter and summer, resulted in seedlings at Mount Connection germinating early in spring or early in autumn having a greater life expectancy than later emergents. Purdie (1977), working in dry eucalypt forest, also found winter and summer to be the times of highest seedling mortality and Grose (1957a), working with high altitude *E. delegatensis*, found that winter mortality was high in seedlings from autumn sowings and that summer mortality was high amongst spring germinants. Similar demographic trends have been identified amongst other plants (e.g. Cook 1980; Gross 1980; Fowler 1988). Laboratory studies in Chapters 4 and 5 indicated a rapid change with age in the drought tolerance and frost resistance of *E. delegatensis*. These ontogenetic changes are almost certainly responsible, in part, for the better survival of early germinants in spring or autumn relative to later emergents.

Early-autumn times of sowing resulted in the majority of seeds germinating prior to the middle of autumn, and sowing in late winter resulted in germination confined almost totally to early spring. Consequently, mortality from these sowing times was low (Fig. 6.13). Late spring or late autumn sowings, with emergence concentrated in the period immediately prior to the periods of high hazard, were particularly unfavourable. This has clear implications for management. Whether seed is sown in the autumn or the spring it is crucial that it is sown early. Natural seedfall in late summer (Grose 1957a) maximises regeneration at these comparatively safe times: the non-dormant seed component germinates early in the autumn and dormant seed early in the spring.

Although some seasons were more hazardous for very young seedlings than were others, each season held its own combination of hazards. This has the resultant effect of diminishing seasonal distinctions in hazard, particularly amongst more established seedlings. Similar compensation among mortality sources has been recorded in other studies (e.g. Sacchi and Price 1992). Nevertheless, it appears necessary to allow for stochastic events, such as severe frosts, to describe adequately survival curves. Looked at over a long time span mortality of tree seedlings has been found to be described satisfactorily by models that allow for a constant rate of mortality (e.g. Harper 1967; Treshow and Harper 1974) or at least models that allow for a relatively gradual transition in the probability of mortality (e.g. Hett and Loucks 1976; West *et al* 1979). However, if the time scale resolution is increased seasonal and chance events become increasingly

significant and more complex models may be required to describe survivorship adequately.

The poor growth of late autumn and early winter emergents relative to spring emergents found in this study has been observed in a number of other studies of eucalypt regeneration (Cunningham 1960, Cremer 1962, Fagg 1981). In each of these instances winters were cold, with a high proportion of frost damage and frost heave to seedlings over-wintering. In this study, seedlings germinating earlier in the autumn at the frostier site, Mount Connection, suffered growth depression more than did seedlings at the Bicheno site. The apical shoot, in particular the naked buds (Webb *et al.* 1983) and leaves not yet fully expanded (see Chapter 5), have been demonstrated in *E. delegatensis* to be, with the exception of cotyledons, the least tolerant of all above ground tissue to frost. Seedlings germinating in the autumn may suffer frost damage to the apical bud during winter and miss the commencement of the growing season in spring. This process has been proposed as one of the principal factors in the occurrence of the growth check syndrome in regenerating stands of *E. delegatensis* (Nunez and Sander 1982; Keenan and Candy 1983; Webb *et al.* 1983).

6.5.3 Conclusion

Unlike many perennial plants, eucalypts, which rely on catastrophic events such as wildfire to prepare a regeneration site, may only get one regeneration opportunity. This is a situation more analogous to annual plants. When a seed commences germination, it is literally gambling its life that the subsequent period will be suitable for establishment. The perpetuation of the tree, however, is less vulnerable than its individual propagules since it can maximise the chances of perpetuation by having seeds that respond differentially to environmental triggers. While severe frost and drought events may occur over the course of much of the year in the climatic range of *E. delegatensis* and *E. amygdalina*, there is a marked seasonality in the probability of occurrence with severe frosts most likely in late autumn and winter, and drought most probable in summer and early autumn. It has been shown that the occurrence of multiple cohorts within years from the one seeding event is favoured by a high year to year variation in the probability of survival of seeds germinating at any one time (Cohen 1968, Venable and Lawlor 1980, Grime 1981, Venable 1985, 1989). Seeds of many species growing in temperate climates typically show a bimodal distribution of germination and it has been further suggested that a higher percentage germination in one cohort is favoured by a greater probability of favourable

conditions for that cohort (Venable 1989). Although natural seedfall of *E. delegatensis* has been recorded in all months of the year, it peaks in autumn (Grose 1957a). In environments with a high probability of killing autumn frosts, we would, therefore, expect a high proportion of seed to exhibit dormancy with a consequently high proportion of spring germinants. The seedlots from different provenances investigated in Chapter 2 displayed considerable variation in the proportion of dormant seed, and this appeared to be related to the coldness of the site from which the seedlot derived. The greater susceptibility of younger seedlings to drought (see Chapter 4) would be expected to act contrary to selection for delayed germination and ensure that at least a proportion of the seed population was not dormant. Compared to other seed germination characteristics the dormancy of seed from trees within a site was relatively uniform (Chapter 3), possibly indicating that over many generations an appropriate dormant to non-dormant seed ratio had evolved.

The slow growth of autumn emergents relative to spring emergents complicates the question of fitness in a given environment. Competition in the first years of life is fierce, and out of approximately 100 000 seeds sown per hectare (and possibly more following natural seedfall after a fire), up to 50 000 seedlings may arise, but perhaps only 500 individuals will remain alive after 10 years. Individuals that fail to maintain a competitive position with respect to adjacent individuals have a greatly reduced chance of reaching reproductive age. Maximum reproductive fitness in a given environment in this case is, therefore, a complex balance determined by the frequency of conditions promoting rapid and complete germination, those causing mortality of seedlings and those giving rise to rapid growth.

In this study regeneration opportunities varied between the years examined. Autumn 1989 at the Bicheno site was favourable for regeneration whereas autumn 1990 was inappropriate. Bowman (1984) investigated the natural regeneration of uneven-aged stands of high altitude *E. delegatensis* forests and found that although widespread germination only occurred following wildfire, due to seasonal variation in the suitability of conditions for seedling survival, not every wildfire event was associated with a regeneration episode. Year-to-year variation in environment has been shown in other studies to have a profound impact on the characteristics of the regeneration niche and population dynamics (e.g. Klemnow and Raynal 1981; Mack and Pyke 1983). This must be considered in the selection of suitable silvicultural regimes and sowing practices for artificial regeneration of *E. delegatensis* forests. If seed regeneration is relied

upon exclusively, the stochastic nature of the regeneration niche and the occurrence of mortality factors means that inevitably there will be years when regeneration fails. It also supports the conclusions from the initial review of research into the time of sowing of seed that extrapolation from experiments with limited replication in time is unsafe. The identification of the most appropriate time of sowing involves not only the estimation of the mean response to particular dates of sowing but also an assessment of the variability, or risk, inherent in different dates. Clearly this is a question that is not easily answered by field experimentation, and is more readily investigated by a modelling approach.

From a modelling perspective, the agreement between predicted limits to germination and observed patterns in the field is encouraging. While some emergence times were more hazardous than others, the compensatory nature of seasonally distributed mortality factors seems to indicate that getting a prolific germination flush may be of prime importance in reafforestation activities. This in many ways simplifies the task and means that predictive modelling of emergence alone may define the optimum sowing time adequately.

Chapter 7: A seed germination model for emergence of a partially dormant seed population under conditions of varying temperature and water potential.

7.1 Introduction

A truly mechanistic model that described germination would most likely be highly complex and virtually impossible to use (Thornley 1986). Highly empirical approaches, at the other extreme, lack the flexibility to deal with dynamic responses to environmental conditions and do not usually provide scientific insight. A middle course is a compartmental analysis. In compartmental analysis, the system is divided into a number of compartments which, in the case of seed germination correspond to seed states. The material flow between these compartments is described by fluxes, in this case as rates of transition of seeds from one state to another. The model is developed as a set of ordinary differential equations, one for the material balance of each compartment (O'Neill 1979a). This approach provides an intermediate modelling approach when inadequate data are available to justify a more complex model. While the model will inevitably be less than adequate, it avoids the sophism of highly complex models developed from insufficient data and in the short term provides immediate predictions for management purposes. As more information becomes available regarding system processes, the empirical factors in the model can be progressively replaced by mechanistic descriptions.

7.2 The germination model

The germination model illustrated (Fig. 7.1) is a simple schema that appears sufficient to account for the full range of temperature and water potential response of *E. delegatensis* seed observed in Chapter two.

It is assumed initially that dry seed (S) requires a discrete time lag (ρ) to imbibe water to become physiologically active. This is consistent with a large body of literature (e.g. Gibson and Bachelard 1988) that indicates metabolic activity is delayed until water content exceeds a threshold value, and that the initial stage of

seed imbibition is a physical process (Mayer and Poljakoff-Mayber 1975; Hegarty 1978). This corresponds to phase I of seed germination (*sensu* Hegarty 1978). Following imbibition, seed is presumed to comprise two populations, those non-dormant and commencing pre-germination processes (**N**) and those that are dormant and will require stratification to remove dormancy (**D**). Seed that is non-dormant (**N**) may become dormant (**D**), and *vice versa*. Given sufficient time, seed develops to become resistant to the induction of dormancy. *Eucalyptus delegatensis* seeds rupture the testa well before radicle emergence, at which stage they may be considered to be irreversibly committed to the emergence process, since they will die if dried (see Chapter 2). These seeds (**G**), are ready to germinate immediately. They may be considered to be of two types (**G1** and **G2**), those able to continue germination at the current level of soil moisture, and those unable to do so. Work suggests that this is the appropriate stage to impose differences in seed response since it is not the level of seed hydration that influences whether a seed will germinate or not (Owen 1952; Hegarty 1978). It has been suggested that the germination process most sensitive to moisture stress is the initiation of cell elongation (Hegarty 1977; Hegarty 1978; Dell'Aquila 1992), so it appears appropriate to discriminate between those seeds that can and can not germinate at this point in the model. There is considerable variation in the ability of individual seeds within a seedlot to germinate at a given level of stress (Hegarty 1978; see also Chapters 2 and 3) so the introduction of a stochastic term at this point appears justified. The ratio of **G1** to **G2**, given in the model by γ, is determined by the ambient conditions and is presumed to change instantaneously.

Movement of seeds between compartments is proportional to the number of seeds in that compartment. Some seeds will be ready to move from one compartment to the other almost instantly, others will require a more prolonged dose of the dormancy-removing or dormancy-inducing factor. The rate variables *k1, k2, k3* and *k4* represent this variation in rate of movement between compartments. These rates are positive unless movement is not possible in which case they are equal to zero. Before seed is observed to germinate growth processes also need to occur. These are modelled as a timelag, τ. This corresponds to the combined second and third phase of the germination phase described by Hegarty (1978) involving the synthesis of organelles and enzymes for catabolism of reserves and the synthesis of new cellular components and radicle emergence.

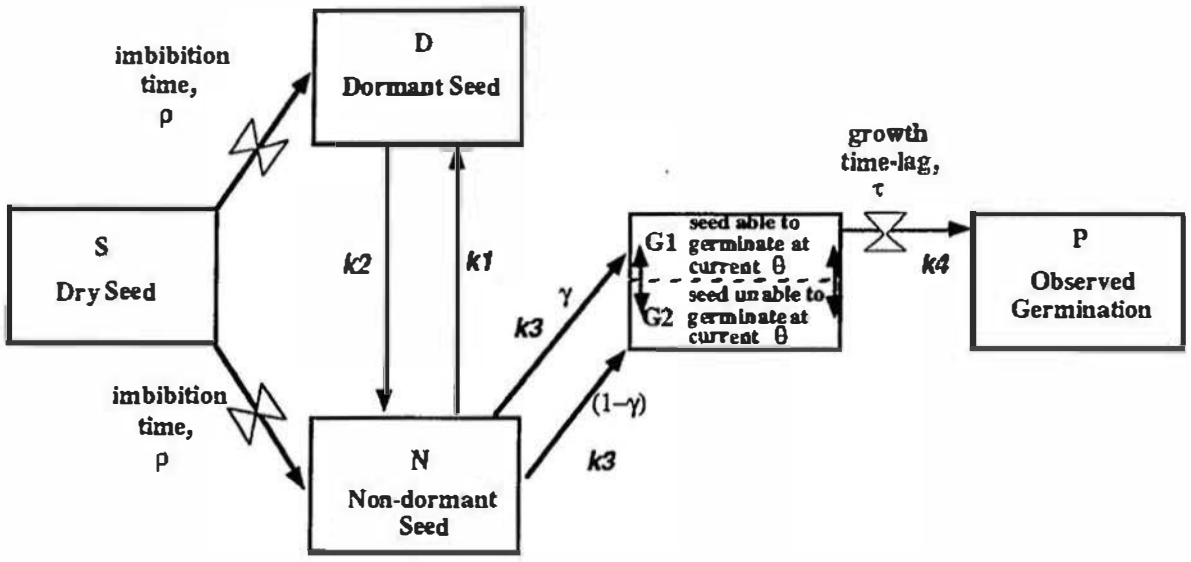


Fig. 7.1. Compartmental model of seed germination. *k1*, *k2*, *k3* and *k4* are rate parameters. γ is the proportion of the remaining seed sample that will germinate at the current water potential. Interchange between compartments **G1** and **G2** is instantaneous with the prevailing soil water potential.

7.3 Mathematical formulation

The system is modelled by the following set of equations:

$\mathbf{D}'(t) = -k2.\mathbf{D}(t)+k1.\mathbf{N}(t)$

$\mathbf{N}'(t) = k2.\mathbf{D}(t)-(k1+k3).\mathbf{N}(t)$

$\mathbf{G1}'(t) = \gamma k3.\mathbf{N}(t)-k4.\mathbf{G1}(t-\tau)$

$\mathbf{G2}'(t) = (1-\gamma)k3.\mathbf{N}(t)$

$\mathbf{P}'(t) = k4.\mathbf{G1}(t-\tau)$

With initial conditions at $t=\rho$:

$\mathbf{D}(\rho) = D_0$

$\mathbf{N}(\rho) = N_0$

$\mathbf{G1}(\rho) = G1_0$

$\mathbf{G2}(\rho) = G2_0$

$\mathbf{P}(\rho) = P_0$

and $D_0+N_0+G2_0+G1_0+P_0 = 1$.

The rates $k1$, $k2$, $k3$ are activated after time $t = \rho$.

The rate $k4$ is activated after time $t = \rho+\tau$.

γ changes instantaneously with conditions.

The embedded time lag, τ, leads to complications in the solutions of the differential equations. The exact solution follows (David Paget, Department of Mathematics, Universtiy of Tasmania pers. comm, see Appendix 4 for full development):

for all $t \geq \rho$,

$$\mathbf{N}(t) = \frac{\alpha A}{k3}.e^{-\alpha(t-\rho)} + \frac{\beta.B}{k3}.e^{-\beta(t-\rho)} \qquad (1)$$

$$\mathbf{D}(t) = (1-\frac{\alpha}{k3}).A.e^{-\alpha(t-\rho)} + (1-\frac{\beta}{k3}).B.e^{-\beta(t-\rho)} \qquad (2)$$

for $\rho+(m-1)\tau \leq t \leq \rho+m\tau$:

$$\mathbf{G1}(t) = \gamma.[X_{m-1}(t) - A\frac{r^m - e^{-ma\tau}}{r-e^{-a\tau}}e^{-\alpha(t-\rho-(m-1)\tau)} - B\frac{s^m - e^{-mb\tau}}{s-e^{-b\tau}}e^{-\beta(t-\rho-(m-1)\tau)}] \qquad (3)$$

$$G2(t)=G2_0+(1-\gamma).[1-P_0-G1_0-G2_0-Ae^{-\alpha(t-\rho)}-Be^{-\beta(t-\rho)}] \quad (4)$$

$$P(t)=1-G1(t)-G2(t)-D(t)-N(t) \quad (5)$$

where:

$p=\frac{k1+k2+k3}{2}$, $q=k2.k3$, $k=\sqrt{p^2-q}$, $\alpha=p+k$, $\beta=p-k$, $r=\frac{k4}{\alpha}$, $s=\frac{k4}{\beta}$,

$$A=\frac{k3.N_0-\beta(1-G1_0-G2_0-P_0)}{2k}, \quad B=\frac{(1-G1_0-G2_0-P_0)\alpha-k3.N_0}{2k},$$

and $X_{m-1}(t)$ is a polynomial of degree m-1 given recursively by:

$$X_i(t)=X_{i-1}(\rho+i.t)+A.r^i+B.s^i - k4\int_{\rho+(i-1)\tau}^{t-\tau} X_{i-1}(u).\,du.$$

with $X_0(t)=A+B+\frac{G1_0}{\gamma}$

This form is too complicated for practical use. Attempts to approximate the solution using a truncated Taylor series or a Laplace transform method fail to mimic the practical situation realistically. The former behaves reasonably at small values of t, but eventually diverges, and the latter results in an oscillating value of G1 and unrealistic constraints on parameter values (e.g., that *k4* must be less than $\frac{\pi}{2}$). The most expedient solution is to allow for the time delay, τ, only up until $t=\rho+\tau$. Provided that τ is small in relation to the total emergence time this should present no substantial problems.

Thus:

$$G1(t)=\frac{\gamma.A.e^{-\alpha(t-\rho)}}{\frac{k4}{\alpha}-1}+\frac{\gamma.B.e^{-\beta(t-\rho)}}{\frac{k4}{\beta}-1}+C1e^{-k4.t} \quad (6)$$

where:

$$C1=\gamma.[1-G2_0-P_0-\frac{A}{1-\frac{\alpha}{k4}}+\frac{B}{1-\frac{\beta}{k4}},]$$

Using these formulae, some of the normal measures of germination performance can be derived.

Gemination capacity is the proportion of seeds observed to germinate as time tends to infinity. Hence:

$GC=P(\infty)$, assuming no seed mortality and a steady state soil water potential

$=1-D(\infty)-G2_0+P_0$, since as $t\rightarrow\infty$, $N,G1\rightarrow 0$,

but if $k2>0$ then as $t\rightarrow\infty$, $D\rightarrow 0$, and hence $P(\infty)\rightarrow\gamma-G2_0+P_0$,

however if $k2=0$, then it can be shown that,

$$P(\infty)=\gamma.[1-(\frac{\beta}{k3}-1).B]-G2_0+P_0 \qquad (7)$$

The mean time to germination is given by:

$$\bar{t}=\int_0^\infty t.\frac{\delta P}{\delta t}.dt$$

which following simplification can be approximated by:

$$\approx\rho+\frac{1}{k4}-\frac{A}{\alpha}-\frac{B}{\beta} \qquad (8)$$

7.4 Estimating parameter values

The model has seven parameters requiring estimation, and many more when these parameters themselves are to be allowed to vary with changing conditions of temperature and water potential. However, the task is simplified since the proportion of seeds in each state over time can be observed or estimated by experimentation and deduction, leaving only a small number to be estimated by non-linear regression methods.

7.4.1 Modelling imbibition (ρ)

The change in relative water content with time can be modelled using the rectangular hyperbola:

$$RWC=\frac{a.b.t}{1+b.t} \qquad (9)$$

where RWC is the relative water content at the end of the time interval t (hours), a is the relative water content of fully imbibed seed, and b is a rate constant. In chapter 2 imbibition rates under a range of conditions of temperature and matric water potential were tested (Fig. 2.15, 2.16, 2.17). Using these data, the parameters that influence the rate of relative water content increase can be specified. Equation (9) was fitted to the imbibition curve for each set of

conditions. It is assumed that a is constant, with a true value of 0.44. A linear relation can be fitted to the response of b to temperature, in degrees celsius:

$$b=0.03.\text{Temperature} \qquad r^2=0.98^* \qquad (10)$$

The response to water potential is best dealt with as a two-stage model. At matric potentials above -0.5 MPa there is no effect, but at matric potentials, ψ, below this:

$$b=0.03.e^{3.00*(\psi+0.5)}.\text{Temperature} \qquad (11)$$

Insertion of (10) and (11) into (9) and transposition, allows calculation of the time lag for imbibition to any chosen level of relative water content, and hence estimation of the time lag, ρ. The form of this model suggests an additive rather than a multiplicative interaction between temperature and water potential on imbibition rate. This is supported by the findings in Table 2.3 which indicated that the interactive effect of the two on germination rate was not significant. A similarly non-significant effect is observed if the time to commencement of germination is considered. A comparison of the predicted and observed relative water content changes with time in response to temperature is given in Fig. 7.2. for the first 24 hours of the imbibition process when relative water content is changeing most rapidly with time. The observed data used in this figure is drawn from the imbibition experiments in Chapter 2.

7.4.2 Modelling radicle growth (τ)

This time lag, τ, may be condsidered to be the sum of the growth processes that precede radicle emergence. In the absence of the timelags the model structure (see the section 7.3) implies that germination commences almost immediately. Therefore, if ρ is known, τ can be estimated as the remainder of the time before the first seed is detected to emerge.

The response of growth processes of this kind to temperature have been modelled successfully using a thermal time approach by a number of workers (e.g. Landsberg 1974; Cannell and Smith 1983; Feng *et al.* 1990). It is assumed that growth is proportional to the number of day degrees accrued. For day degrees to accrue, the temperature must be above a certain minimum required for

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* r^2 adjusted for no-intercept model. See SAS Institute Inc. (1989) for fuller explanation.

metabolic activity. Pre-treatments such as stratification may shorten the time for emergence at test conditions due to the day-degrees accrued during pre-treatment. This was observed in chapter 2 when it was noted that after 56 days stratification at 5°C germination had commenced. The following thermal time model was fitted to the distribution of the emergence times from experiments conducted in chapter 2 (minus the estimated value of ρ from (9, 10 and 11)). These data are drawn from the tests illustrated in Fig. 2.2.

$$\tau=\frac{\hat{T}-(5-T_{min})*\theta}{T-T_{min}}, \qquad (12)$$

where $\hat{T}$=the required day degrees to the commencement of emergence, T_{min}=the minimal basal temperature for growth processes, θ=the duration of stratification in days, T=the temperature in degrees celsius.

The estimates and 95% confidence intervals obtained for the test data were: $\hat{T}$=118.29 (103.80-132.81) and T_{min}=2.89 (2.48-3.10). Growth response is probably the most sensitive of all germination processes to water potential (Hegarty 1977; Hegarty 1978; Dell'Aquila 1992) and it is assumed that changes in germination rate are acting via τ and k4. At matric potentials of -0.1 MPa or more the rate of germination is unaffected, but at lower matric potentials there is an exponential decline (see Chapter 2, Fig. 2.9, 2.10 [*nb.* log. scale used in figure]). For matric potentials below -0.1 MPa the growth time lag can be represented by:

$$\tau=\tau_o.e^{-1.07.\Psi} \qquad \text{where } \tau_o \text{ is the value of } \tau \text{ when } \psi=0 \qquad (13)$$

A plot of predicted and actual values of time till emergence for seed samples under various temperature and stratification treatments are given in Fig. 7.3. The observed responses are drawn from experiments conducted in Chapter 2.

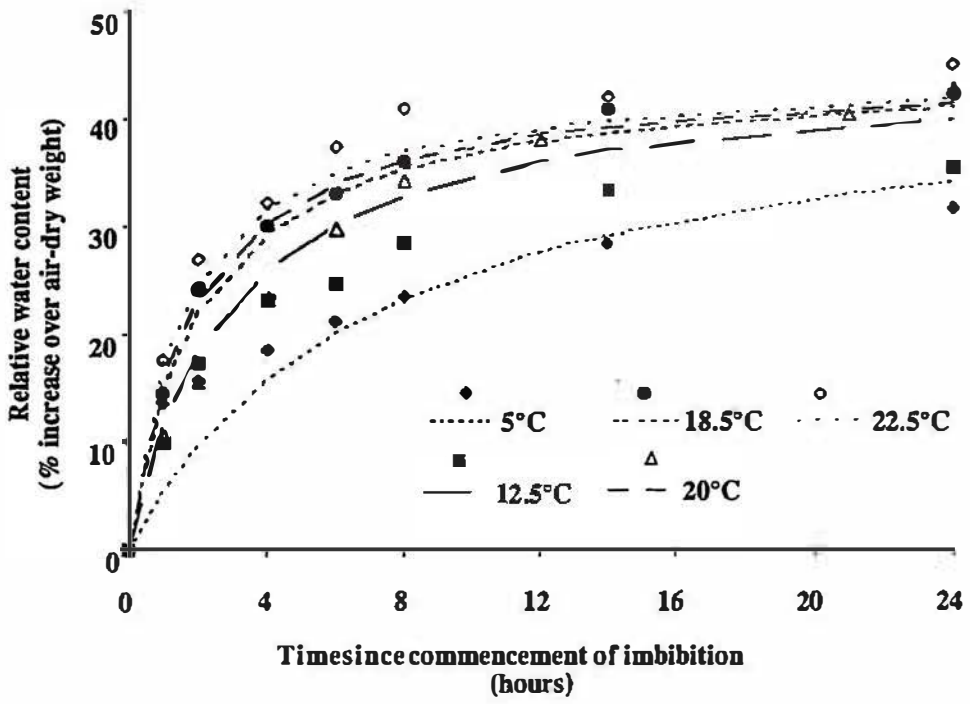

Fig. 7.2. Predicted and observed relative water contents (% increase in weight over air-dried weight) during the first 24 hours of imbibition at a range of test temperatures. The symbols are the mean values of the observed relative water contents from experiments conducted in chapter 2 and the lines represent the predicted values using formula (9).

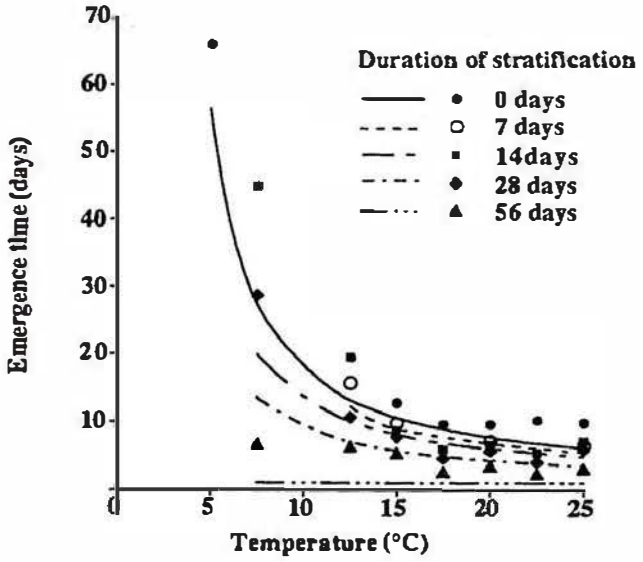

Fig. 7.3. Observed and predicted emergence times following different periods of stratification. The symbols represent observed, and lines predicted, times for the first ten percent of a seed sample to germinate.

7.4.3 Modelling the rate parameters (k1, k2, k3, k4)

For any set of data there can be found a combination of values of *k1, k2, k3* and *k4* that best fit the observations. However, there will exist numerous other sets of these parameters that result in an almost equivalent goodnes of fit to the data. It is assumed *a priori* that stratification does not alter the rate parameters (as per Grose 1963), only the values of D_o, N_o, G_o and P_o and the timelags, ρ (since imbibition has occurred during stratification) and τ (since growth processes, and ultimately germination, occurs at stratifying temperatures). Past research into the use of compartmental models to simulate seed germination has reported good results when the Arrhenius function has been fitted to the rate of seed movement between compartments (e.g. Johnson and Thornley 1985; Hasgeth and Cody 1993). The Arrhenius function is used in this research to simulate the response of the various rate parameters in the model to temperature. The function for **P**(t) can assume a wide range of forms, and a set of parameters could be found that would give almost perfect congruence between predicted and actual cumulative germination curves. However, the objective of this modelling exercise is to observe whether a set of parameters consistent with the *a priori* assumptions regarding the form of the response surface to temperature and water stress can be found that gives a generally satisfactory correspondence to the observed responses.

The easiest of the rate parameters to estimate is *k4*. After seed has been stratified for 56 days it is relatively insensitive to dormancy-inducing temperatures. In terms of the assumptions of the model this implies that all, or nearly all, seeds have progressed to the **G** compartment. Hence the rate at which emergence occurs is virtually entirely a function of *k4*. These rates were calculated by non-linear regression means (NLIN method=DUD SAS (1989)) for the germination tests conducted over a range of temperatures that were reported in chapter 2, setting G_o to 0.9. The best fit estimates of *k4* are given in Fig. 7.4. The response *k4* to temperature is similar in form to those processes described by the modified Arrhenius function of Johnson and Thornley (1985). These authors combined the Arrhenius function with the Boltzmann distribution (which defines the number of enzyme molecules in the active and inactive states) to describe enzymic reactions with a temperature optimum and where the enzyme can exist in two forms (an inactive and an active form), giving the expression:

$$k = \frac{a\,e^{-b/T}}{(1+e^{c-d/T})}, \qquad (14)$$

where k=the rate constant, T=temperature, a=a constant, which can be viewed as the maximum rate of reaction when there is no activation energy difference between reactant and product, b=Ea/R where Ea is the activation energy of the enzyme and R is the gas constant, c=δS/R where δS is the entropy difference between the active and inactive states and where d=δH/R where δH is the enthalpy difference between the active and the inactive state.

Feng *et al.* (1990) re-paramaterised this equation to include the optimum temperature, T_{opt}, a more appropriate, and easily verified, parameter than entropy and enthalpy change. At the temperature optimum δk/δt=0, so that:

$$Topt = \frac{d}{c+\ln(d/b - 1)}, \qquad (15)$$

and hence, substituting (15) into (14),

$$k = \frac{ae^{-b/T}}{1+me^{d(1/Topt-1/T)}}, \qquad \text{where } m = \frac{b}{d-b}. \qquad (16)$$

However, in this paramaterization a and b are highly correlated, a fact little considered by workers who have assigned physiological interpretation to these parameters after having judiciously decided which parameter should be allowed to vary (e.g. Feng *et al.* 1990). This correlation also inevitably leads to poor convergence in function solution and leads to both parameters being poorly estimated. A further re-paramaterization, can be made to relieve this slightly (Steve Candy, pers. comm.):

$$\text{at } T=T_{opt} \qquad k_{opt} = \frac{ae^{-b/Topt}}{1+m}, \text{ where } k_{opt} \text{ is the maximum rate.} \qquad (17)$$

Therefore

$$a = k_{opt}e^{b/Topt}(1+m), \qquad (18)$$

and hence

$$k=\frac{k_{opt}e^{b(1/Topt-1/T)}(1+m)}{1+me^{d(1/Topt-1/T)}} \qquad (19)$$

The parameter estimates and the 95% confidence interval asymptotic standard errors are k_{opt}=0.22 (0.22), b=88.88 (49.05), d=151.64 (32.37) and T_{opt}=20.29 (1.00).

As previously for the parameter τ, the decrease in germination at water potentials below -0.1 MPa can be modelled with an exponential function:

$k4=k4_0e^{2.75.\Psi}$ where $k4_0$ is the value of k4 when ψ=0 (20)

The parameters k1 and k3 were estimated by assuming *k2* to be approximately equal to zero for temperatures above 5°C, and finding the least squares solution to the non-linear regression of **P**(t) using the data from the temperature experiments reported in chapter 2. It is assumed that if the relative water content of the seed exceeds 40%, or a water potential of approximately -3.0 MPa (Gibson and Bachelard 1988), these rates are unaffected.

The parameter *k3* was then modelled using equation (19). Best fit parameters and the asymptotic standard errors were k_{opt}=0.10 (0.01) b=3.78 (1.58) d=1904.00 (853.00) and T_{opt}=20.76 (1.19).

The parameter *k1* was modelled as the sum of cold temperature inhibition and high temperature inhibition, using the following equation:

k1=high temperature inhibition + cold temperature inhibition
if t < 15°C $k1 = a-b.T+ce^{-d/T}$
if t ≥ 15°C $k1= ce^{-d/T}$,where T is the ambient temperature °C. (21)

The best fit parameters and asymptotic standard errors are: a=0.59 (0.01), b=0.040 (0.001) c=6.24 (2.19) d=108.50 (8.28).

The fit of *k1*, *k3* and *k4* to the above functions is shown in Fig. 7.4.

In the conceptual schema (see Fig. 7.1 and section 7.3) seed in the G compartment is not susceptible to dormancy. The minimum germination

capacity observed following different durations of stratification can therefore be used as an indication of the proportion of seed in G at the termination of stratification, since, provided that *k4* is greater than zero, all seed in the G compartment will ultimately germinate. At 25°C the rate of dormancy induction, *k1*, is high relative to the rate at which seeds move from the N to G compartments and the final germination capacity that is observed is a close reflection of the quantity of seed commencing in compartment G. Therefore, by stratifying seed for different lengths of time and allowing them to germinate at 25°C, the change in G with stratification period can be estimated. This in turn allows for the estimation of the rate of dormancy removal by the stratification process, *k2*. Experiments in which seeds were allowed to germinate at different temperatures following stratification for a sufficient time to render seed non-dormant are reported in chapter 2 (illustrated in Fig 2.5 and Fig. 2.6). The data from these tests were used to find a value of *k2* that gave the best fit to the germination capacities observed at the different germination temperatures.

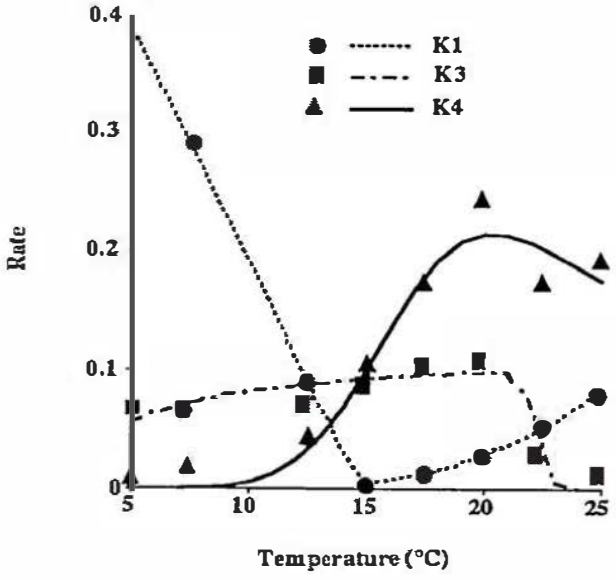

Fig. 7.4. Observed (i.e., best-fit parameter values) (symbols) and modelled parameter responses (lines) over the range of test temperatures.

7.5 Results And Discussion

The evaluation and verification of a model involves consideration of its simplicity, the appropriateness of its formulation relative to what is known about system functioning, the accuracy with which it fits the developmental data, the sensitivity of the model to variations in parameter estimates, and, perhaps most critically, its ability to accurately simulate the outcomes from input data sets independant to those used for model development.

7.5.1 Model simplicity and appropriateness

The conceptual schema (Fig. 7.1) that forms the basis of the model is simple. However, even relatively simple models can give rise to complex mathematics. Because seed germination and temperature responses are predominantly sigmoidal, exponential or asymptotic in form, non-linear models inevitably arise. The conceptual schema proposed here, with its embedded time-lag, causes further mathematical complexity because the calculus of time-lags is complex (Driver 1977). However, non-linear responses and time-lags are both observed biological phenomena and must be accounted for in mechanistic biological models. The question of simplicity can only be reconciled by choosing the level at which the model is to be evaluated. The model described here is intuitively appropriate, easily understood and consistent with current theory at the conceptual level, but it is mathematically difficult. It is at the conceptual level that the appropriateness of the model is best decided, since the mathematics is merely a translation of these concepts into formulae. It is unlikely that the complexity of behaviour of a seed population exhibiting reversible dormancy can be explained by a simpler model than represented in Fig. 7.1. In addition the model formulation provides the opportunity to easily estimate three of the parameters (r, t and *k4*) using empirical means.

The phrasing of the sub-models controlling transfer rates is a matter of preference, although for simplicity in this paper existing mathematical expressions for the sub-model processes have been used or adapted where possible [e.g. the monomolecular function for imbibition of Dewez (1964) and Blacklow (1972); the thermal sum approach to growth of Landsberg (1974), Cannell and Smith (1983) and Feng *et al.* (1990); and the Arrhenius function approach to modelling enzymic reactivity of Johnson and Thornley (1985) and the modelling of rates in compartmental analysis of Thornley 1986 and Hasgeth

and Cody 1993]. Where possible the actual mathematical expressions chosen have been those for which physiological support is strongest.

Probably the least justifiable assumption made in the modelling process is that the relationship between seed germination and temperature, and indeed time, is deterministic. Seeds are biological entities for which variation seems a fundamental attribute, and they can only be deterministic in terms of some mean response. A more realistic and appropriate means of analysing germination may be to take naturally-occurring variation into consideration, and undertake some form of stochastic modelling (e.g., Matis and Wehrly 1979). Deterministic models, however, can be justified on the pragmatic grounds of being simpler analytically and, even if not fully realistic, of being more readily compared to available data (O'Neill 1979b; Poole 1979).

7.5.2 Model accuracy

Model accuracy is used here to refer to the fidelity with which the developed model fits the data used in its development. The predicted and actual germination capacities with regards to temperature and stratification are shown in Figs.7. 5a and 7.5b. The inconsistency in fit at low temperatures and following long stratification periods may be a result in part of premature truncation of the germination test and the loss of seeds as a result of fungal decay during the protracted test period. The model accurately predicts germination rate (using $1/t_{50}$) over most of the range of temperature and stratification periods (Fig. 7.6). It is poorest in prediction at intermediate stratification periods. Nevertheless the response of germination rate is predicted accurately. A typical fit to the experimental data is shown for the 20°C data in Fig. 7.7. The model predicts accurately the germination capacity across the range of stratification periods tested. The precise forms of germination curves is less well defined. The predicted and actual responses to soil water potential are given in Fig. 7.8 and the response interaction of temperature and water potential in Fig. 7.9.

The model output is reasonably robust to variation in parameter values. Table 7.1 lists the affect of varying each parameter by ±50% of the estimated true value on germination capacity and germination rate. In the most severe case, this is reflected in a 32% error in germination rate and an 18% error in germination capacity. Variation in the parameter $k2$ will only become significant at stratifying temperatures. Variation in $k4$ will cause a greater percentage error after seed has

been stratified for long periods and the effect of *k3* on germination rate is diminished.

Table 7.1. The effect of varying parameter values by ±50% on measurements of germination performance, the germination capacity (GC) and the time taken to reach 50% of the germination capacity (*t50*) at 20°C.

Parameter	Percentage Error			
	+50%		-50%	
	GC	*t50*	GC	*t50*
k1	6	<1	9	3
k2	0	<1	0	<1
k3	7	11	18	28
k4	0	7	0	32
τ	0	25	0	25
ρ	0	<1	0	<1

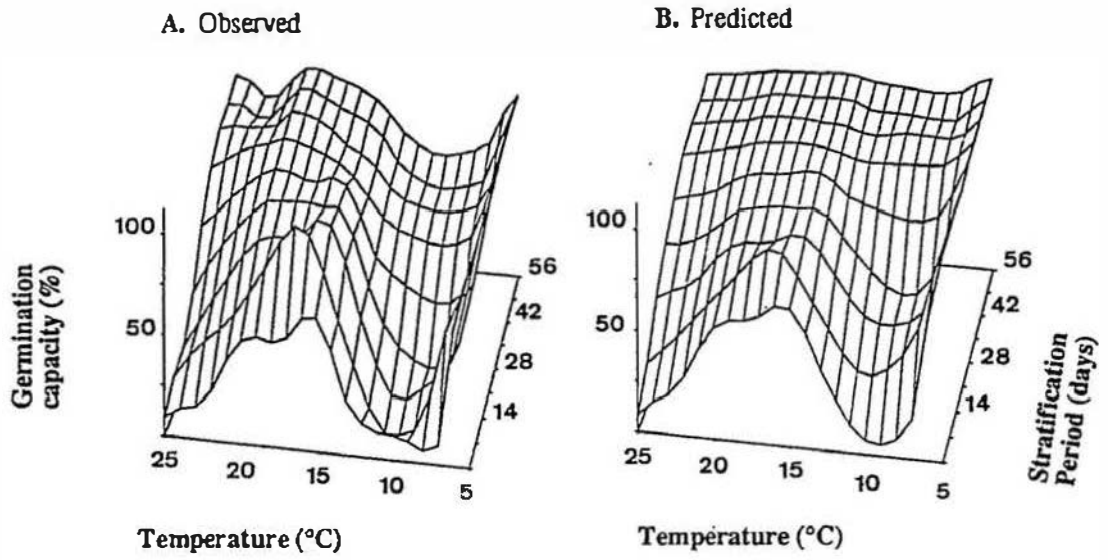

Fig. 7.5. Observed and predicted germination capacity response to temperature and stratification period.

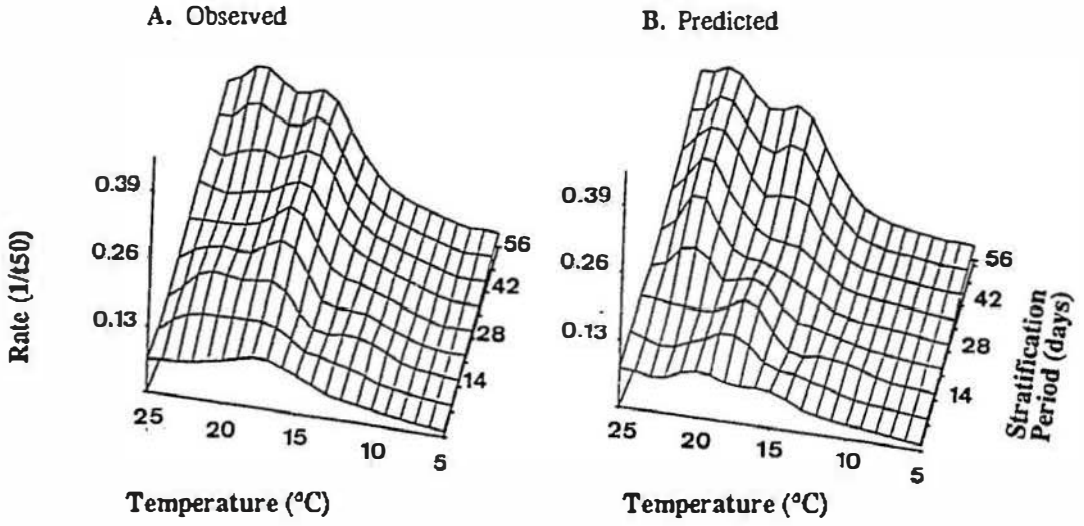

Fig. 7.6. Observed and predicted germination rate response to temperature and stratification period.

7.5.3 Model Verification

To test the models ability to simulate germination under variable temperature conditions seed was germinated under continuously-moist conditions in a glasshouse where temperatures varied between 8°C and 35°C as well as in a shadehouse where temperatures varied between 2°C and 25°C (using the code in Appendix 6 but with seedloss parameters set to zero and setting the substrate water potential to zero). Conditions of germination were the same as those used to derive the model development data (see Chapter 2). Fig. 7.10 shows the predicted versus actual cumulative germination curves under the respective sets of conditions. The model predicts well the commencement of germination, the rate of germination and the germination capacity. It should be noted, however, that even though the shadehouse temperatures dropped into the range of stratifying temperatures, this was generally for short periods. The shadehouse seeds received temperatures of 5°C or lower for the equivalent of one day spread over a total of six nights, and, as can be seen from Fig. 7.7, the model is poorest in prediction for seeds germinating after moderate periods of stratification (14-28 days). Nevertheless the model seems quite useful for the prediction of seed response to variable temperature under continuously wet conditions.

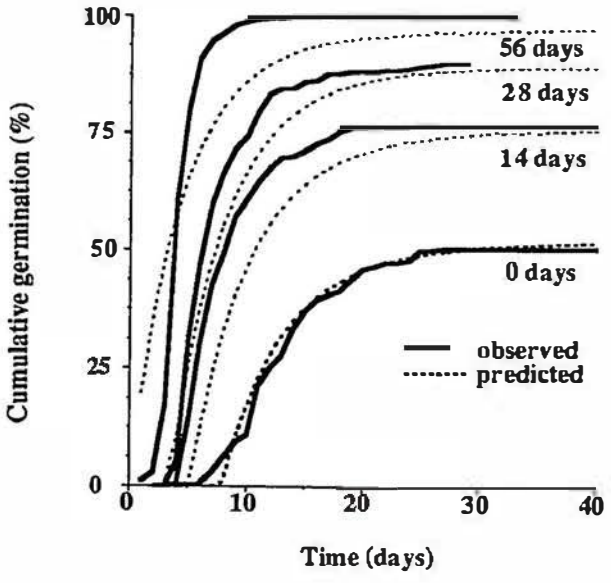

Fig. 7.7. Predicted and actual cumulative germination curves at 20°C following various periods of stratification.

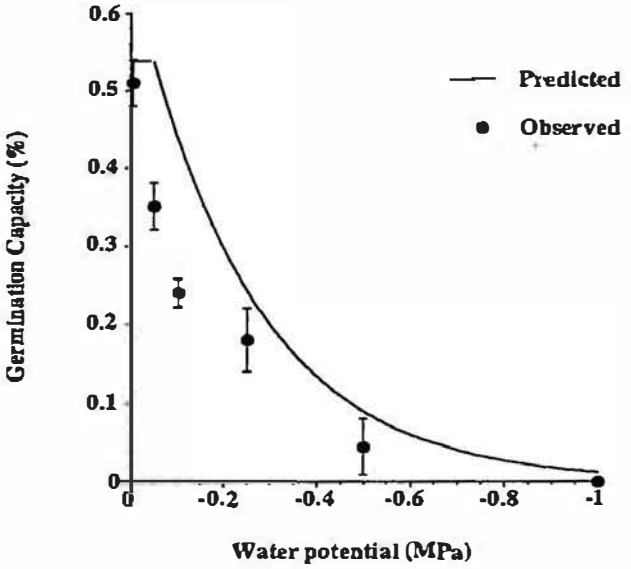

Fig. 7.8. Predicted and observed germination capacity at various levels of water stress. Error bars show the 95% confidence interval of the mean observed value.

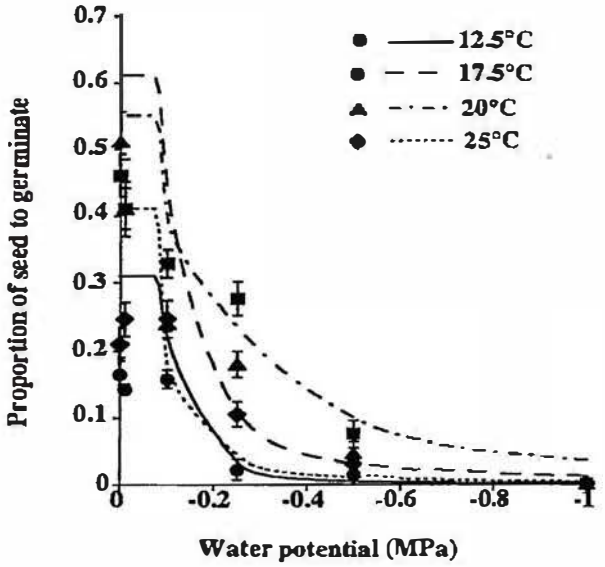

Fig. 7.9. The predicted (lines) and observed (symbol) interaction of temperature and water potential on the germination capacity of non-stratified *Eucalyptus delegatensis* seed. Error bars are the 95% confidence interval of the mean observed value.

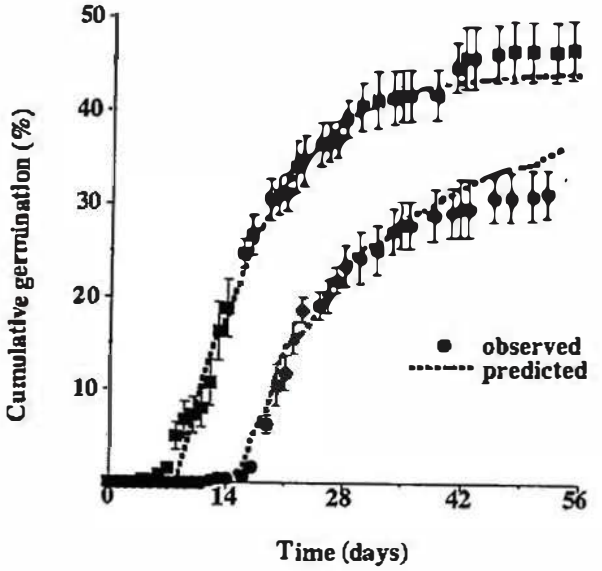

Fig. 7.10. Predicted and actual cumulative germination curves for seeds germinating under semi-conrolled conditions. Error bars are the 95% confidence interval of the mean observed value.

It was assumed in model development that once seeds had progressed to the **G** compartment they would be killed if dehydrated. By calculating the proportion in this compartment, or beyond, over time the predicted impact of dehydration on germination capacity and the actual impact can be compared (Fig. 7.11). The figure indicates that the model provides a realistic representation of the proportion of seed that may be lost due to dehydration during the germination process. The observed data used in this figure is the same as illustrated in Fig. 2.18.

The generality of the model was ascertained by using the model to predict the germination response of seedlots collected from disparate provenances. The seedlots used differed significantly in their temperature and dormancy response (see Chapter 2). The model robustness was tested by observing the degree of manipulation of model parameters required to obtain satisfactory fits for these different provenances. As previously assumed, the proportion of dormant seeds was set to the minimum dormant proportion observed in the germination tests without stratification, ranging from 0% for provenance L17 to 70% for provenance M50. The time-lags and the rate parameters were assumed to be the same as used for the seedlot (M36 from Battaglia 1993) used in the model development. The fit of the model to the germination cumulative germination curves at 20°C is shown in Fig. 7.12. It can be seen that the germination capacity is accurately predicted. The time to the commencement of germination ($\rho+\tau$), however, is not. Extrapolation of the model to other seedlots, therefore, may necessitate an allowance for provenance differences in the time to germination initiation. It can be seen that if this error is corrected, by translating curves along the time axis, that the shape of germination curves is mimicked accurately.

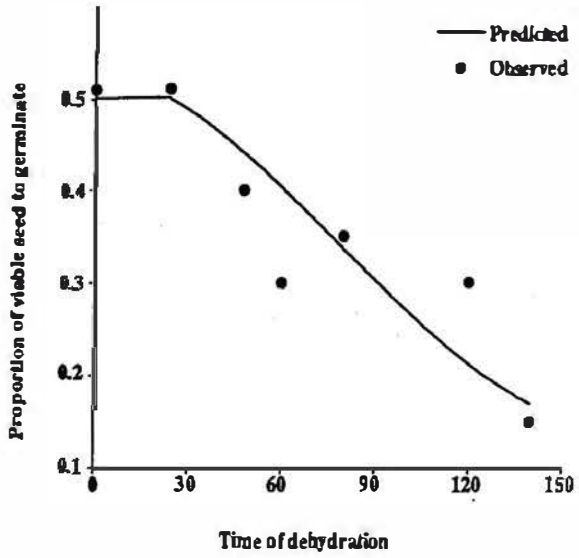

Fig. 7.11. Predicted and observed effects of dehydration during imbibition.

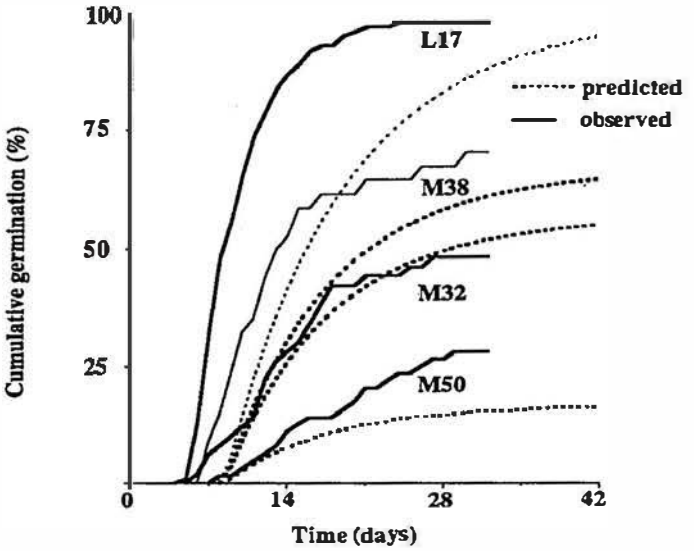

Fig. 7.12. Predicted and actual cumulative germination curves for the germination of seedlots from different provenances. The parameters used for the seedlot for which the model was developed are used throughout and only the initial dormancy conditions have been changed to match the experimentally observed seed dormancy of each provenance.

7.6 Conclusion

The model developed in this paper describes the response of germination after various durations of stratification, and under various regimes of temperature and water potential with reasonable accuracy. In comparison to other germination models developed (e.g. Brown and Mayer 1988; Gracia-Huidobro, Monteith and Squire 1982; Thornley 1986) the model is complex. However, unlike previous modelling exercises, the model described here has considerably more generality: it describes the germination rate and germination capacity response of a partially dormant species to conditions of changing temperature and water potential and makes provision for reversible dormancy. Whether the compartmental schema indicated in Fig. 1 relates to the underlying physiology of germination is a moot point. In the area of seed germination physiology, as in many areas of biology, there is still a paucity of information regarding what underlying regularities exist. In such cases it is reasonable to develop, to some extent *a priori*, a model of the system, to analyse it, and to seek confirmation from field or laboratory data. Such a representation provides a framework for hypothesis generation and testing, and a means of integrating, in a quantitative form, our knowledge about germination processes. There is little doubt that a less empirical and a more physiologically based approach to the lower level processes of seed germination than was undertaken in this paper would greatly improve the applicability of the germination model. Such mechanistic sub-models, as they are developed, can easily be incorporated into the schema indicated here at a future date. Purely empirical models, in contrast, provide little potential for future development and extension into new situations.

Chapter 8: Predicting Field Emergence

8.1 Introduction

While a number of models have been developed to predict the response of some aspect of germination to one, or occasionally two, types of steady-state environmental stimuli with some success, precise prediction of germination response in a variable environment has proven more elusive. Conditions in the surface soil where most seeds germinate, are prone to rapid fluctuations in conditions. Light, temperature and moisture are normally considered the principal environmental determinants of germination (Bewley and Black 1982). Light, within the range that affects germination, remains relatively constant in the field from day to day, although light quality and quantity may change on a seasonal time frame (Monteith 1973), and in a stochastic manner as a result of soil disturbance events or due to canopy disruption. Temperature and soil moisture, however, change rapidly in response to daily, even hourly, weather conditions. It is under these variable conditions that the agriculturalist or forester is most keenly interested in the germination response; the practitioner seeks to know the probability of establishment at different times of sowing in the field where environmental conditions at the germination site are relatively uncontrollable but where the likelihood of different weather sequences can be determined from long term weather records. Provided long-term weather records are available, or can be simulated, the cumulative probability of model outputs can be calculated, and subsequently used for risk analysis (Ritchie 1985; Jones and O'Toole 1986).

Prediction of emergence in the field involves complexities avoided in glasshouse or controlled-environment experiments. In controlled-environment experiments the conditions experienced by the seed can be easily quantified over time. In the field conditions at the soil surface, particularly soil moisture, are more difficult to characterise. Furthermore, in a petri dish each seed can be presumed to be experiencing similar conditions. By contrast, in the field seedbed heterogeneity affects germination performance over local scales, and field emergence is accordingly more temporally variable than is performance under controlled environments (see Chapter 4). Finally, in controlled environment experiments all viable seeds can be expected to germinate given appropriate environmental stimulus, but in the field seed is removed from the soil seedbank by factors such

as predation, fungal attack and burial and cumulative percentage germination will be less than 100% (see Fig. 1.1).

This chapter extends the germination model developed in Chapter 7 to explain the time course of emergence in the field. A model is developed to predict soil moisture conditions. The germination model developed in Chapter 7 is used to predict the timing of emergence. Assumptions are made regarding the rate of attrition of seed from the seedbank, and parameters selected to optimise the fit of observed and predicted cumulative germination curves.

8.2 Predicting conditions at the soil surface

For the two experimental sites described in Chapter 6 soil surface temperature and soil moisture were calculated using a model based on the physically based numerical model of the Philip and de Vries (1957) type. This model form assumes that the processes of water and energy transfer at and near a horizontally homogeneous bare surface can be described by a number of one-dimensional equations. The movement of water is in response to gradients in water potential mediated by hydraulic conductivities in the liquid and vapour phases which vary with soil water content, texture and temperature gradients. Inputs were solar irradiance [estimated from global irradiance and sunrise and sunset times from Beer (1990) and converted to net irradiance using the algorithm of Nunez (1983)], cloud cover (estimated from 3 hourly records from Swansea and Bicheno meteorological stations), wind speed, rainfall and air temperature collected using on site data loggers and humidity (estimated from 3 hourly records from Launceston and Hobart airports). The moisture flux and heat flux were calculated using the following formulae (the origin of formulae are indicated):

$$\frac{\delta\theta}{\delta t} = -K_{w.\Psi}\frac{\delta\psi}{\delta z} - K_{w.T}\frac{\delta T}{\delta z}, \qquad \text{(McInnes } et\ al.\text{ 1986)}$$

$$\frac{\delta T}{\delta t} = -K_{h.T}\frac{\delta T}{\delta z}, \qquad \text{(Stathers } et\ al.\text{ 1985)}$$

where θ is the water content (kg kg^{-1}), z is depth (m), $K_{w.\Psi}$ and $K_{w.T}$ are the hydraulic conductivities with respect to water potential and temperature gradients respectively and $K_{h.T}$ is the thermal conductivity with respect to a temperature gradient. The conductivities were calculated using:

$$K_{w.\Psi}=K_{w.\Psi.liq}+\frac{D_{va}.\alpha.f_a.\rho'_v.h.M_w}{RT}$$ (McInnes *et al.* 1986)

$$K_{w.T}=D_{va}.\alpha.f_a.h.\frac{\delta\rho'_v}{\delta T}$$ (Hammel *et al.* 1981)

where D_{va} is the diffusion coefficient of water vapour in air, α a tortuosity factor (see below), f_a is the effective soil porosity (assumed 0.4), ρ'_v is the saturated vapour density, h the relative humidity, M_w the molecular weight of water (kg mol^{-1}), R the gas constant (461.5 J $kg^{-1}K^{-1}$) and T the temperature (K).

$K_{w.\Psi.liq}$ (m s^{-1})is the liquid hydraulic conductivity, which for a clay loam soil can be approximated by the empirical function (Gardner 1959):

$$K_{w.\Psi.liq}=4\ E\text{-}11.e^{24.VVW},$$

where VVW is the volumetric water content of the soil (kg/kg).

The function used to obtain the vapour diffusion coefficient in air as a function of temperature is (Monteith 1973):

$$D_{va}=2.12\times10^{-5}(1+0.007.T)$$

The saturated vapour pressure of the atmosphere is given by (Hammel *et al.* 1981):

$$\rho'_v=\frac{1.323e^{[17.27(T-273)/(T-35.7)]}}{T}$$

The humidity of the soil air space is given by (Camillo *et al.* 1983):

$$h=e^{(\Psi.g)/R.T}$$

The tortuosity of soil varies approximately linear with moisture content, and for a clay loam can be approximated by (Jackson *et al.* 1974):

$$\alpha=0.3-0.77.VVW$$

The thermal conductivity varies with soil water and was approximated for the clay loam soil studied by the empirical function:

$$K_{h.T} = 0.25+2.134.VVW^{0.516}$$

The temperature flux at the surface was calculated using the method and formulation of Stathers *et al.* (1985) based on the surface energy balance equation:

$$R_s(1\text{-albedo})+\varepsilon_s(R_L-\sigma T_s^4)-H-LE-G_o=0$$

where R_s is the solar irradiance (W m^{-2}), ε_s is the emissivity of the surface (assumed 0.95), R_L is the long wave irradiance from the sky (W m^{-2}), σ is the Stefan-Boltzmann constant ($5.67*10^{-8}$ W m^{-2} K^{-4}), T_s is the absolute radiometric temperature of the surface (K), LE is the latent heat flux density, H is the sensible heat flux density and G_o is the soil heat flux density. The albedo of the soil surface was assumed to be 0.16.

The evaporation from the surface was calculated using (Campbell 1977):

$$Evapo=\frac{\rho_{vs}-\rho_{va}}{r.\rho_w}$$

where ρ_{vs} and ρ_{va} are the vapour concentrations at surface and of the atmosphere, r is the boundary layer aerodynamic resistance and ρ_w is the density of liquid water.

The top 20 cm of soil was divided into 10 layers of increasing thickness, commencing at 1 cm and ending at 5 cm thickness. The model was run with a 60 sec time step. Ambient atmospheric conditions were derived by linear interpolation between known points. Rainfall events were allowed for by bringing successive soil layers up to -0.01 MPa (equivalent to a volumetric water content of 0.22 kg kg^{-1}, which was estimated as field capacity) in the manner of McInnes *et al.* (1986). Parameters for thermal and hydraulic conductivity, specific heat and albedo were taken as typical values for a clay loam (Taylor 1972). Temperatures at the bottom of the soil profile (20 cm) were set to the 9 am and 3 pm readings of soil at 20 cm measured at the nearby Meteorological station at Swansea. Soil at this depth was assumed to stay at field capacity (0.22 kg kg^{-1}). The program is listed in Appendix 5. Units follow the m.k.s. system: energy

is in joules, temperature in degrees kelvin, water potentials are in J kg^{-1} and vapour concentrations in kg m^{-3}.

Estimated and observed values of surface volumetric water content are given in Fig. 8.1 and Table 8.1. Typical predicted diurnal patterns of soil temperature and soil moisture are given in Fig 8.2. The model results are generally indicative of soil moisture conditions. Of the 14 dates for which a comparison between observed and predicted results are made, five of the estimates at the Bicheno site and two of the estimates from the Mount Connection site are poor. The other 20 estimates are indicative of measured site conditions. Large differences between observed and predicted values are confined to the autumn and spring-early summer periods where conditions are varying most rapidly and assumptions about soil moisture at the bottom of the profile are most likely to be in error. The diurnal fluctuations in conditions, although not verified in this study, are consistent with patterns identified in intensive studies (e.g. Hammel *et al.* 1981; Camillo *et al.* 1983; Stathers *et al.* 1985; McInnes *et al.* 1986).

8.3 Spatial heterogeneity in seedbeds

Twelve 1 m^2 plots were located randomly on the seedbed prepared at the Bicheno site. Each plot was divided into twenty five 400 cm^2 sub-plots. The microtopography of each sub-plot was identified as either, hillock, flat or depression. Hillocks comprised 31% (±5%), flat areas 29% (±7%) and depressions 39% (±4%) of the seedbed sampled. Over time seedbed microtopography is 'eroded' and seedbeds become increasingly uniform (Fig. 8.3). In Chapter 4 hillocks were found to be unfavourable microsites, depressions favourable microsites with flat areas intermediate in performance. While this may be a result of a number of factors, soil moisture fluctuation was thought to be a key element of this microsite variability.

Spatial heterogeneity was considered in the modelling process by assuming that microsites experienced one of three moisture regimes. The safest sites were assumed to have a soil moisture corresponding to 3 cm below the soil surface. This means that they are buffered from diurnal fluctuations and dry out more slowly between rainfall events. Microscale depressions and areas shaded by logging residue, or seed that falls into soil crevices and becomes partially buried might be buffered in this manner. An intermediate category of site was considered to be 1 cm below soil surface and the least favourable site resting upon the surface and fluctuating in moisture regime with surface soil. For subsequent

Table 8.1. Observed and predicted volumetric water contents (kg/kg) for the two experimental sites. The maximum and minimum indicate the driest and moistest soil samples at each time.

Date	Bicheno					Mount Connection				
	Pred.	Mean Obs.	SE 95%	Max	Min	Pred.	Mean Obs.	SE 95%	Max	Min
10-Mar-89	0.186	0.164	0.004	0.181	0.150	0.143	0.149	0.007	0.176	0.123
22-Mar-89	0.086	0.094	0.011	0.119	0.070	0.077	0.079	0.002	0.091	0.072
19-Apr-89	0.107	0.164	0.005	0.181	0.140	0.109	0.156	0.003	0.176	0.148
3-May-89	0.181	0.168	0.008	0.199	0.154	0.115	0.137	0.005	0.150	0.128
17-May-89	0.200	0.149	0.004	0.164	0.141	0.190	0.138	0.001	0.141	0.136
1-Jun-89	0.196	0.148	0.002	0.154	0.144	0.178	0.155	0.003	0.164	0.150
21-Jun-89	0.216	0.200	0.001	0.211	0.191	0.230	0.125	0.004	0.236	0.216
26-Oct-89	0.110	0.139	0.006	0.150	0.128	0.190	0.157	0.003	0.161	0.147
13-Nov-89	0.057	0.120	0.001	0.125	0.118	0.053	0.051	0.000	0.051	0.051
6-Dec-89	0.085	0.09	0.005	0.154	0.127	0.115	0.051	0.000	0.051	0.051
18-Dec-89	0.047	0.051	0	0.051	0.051	0.045	-	-	-	-
11-Jan-90	0.056	0.051	0	0.051	0.051	0.044	0.051	0.000	0.051	0.051
6-Feb-90	0.208	0.152	0.005	0.159	0.141	0.161	0.138	0.003	0.145	0.124
22-Mar-90	0.061	0.051	0	0.051	0.051	0.056	0.051	0.000	0.051	0.051

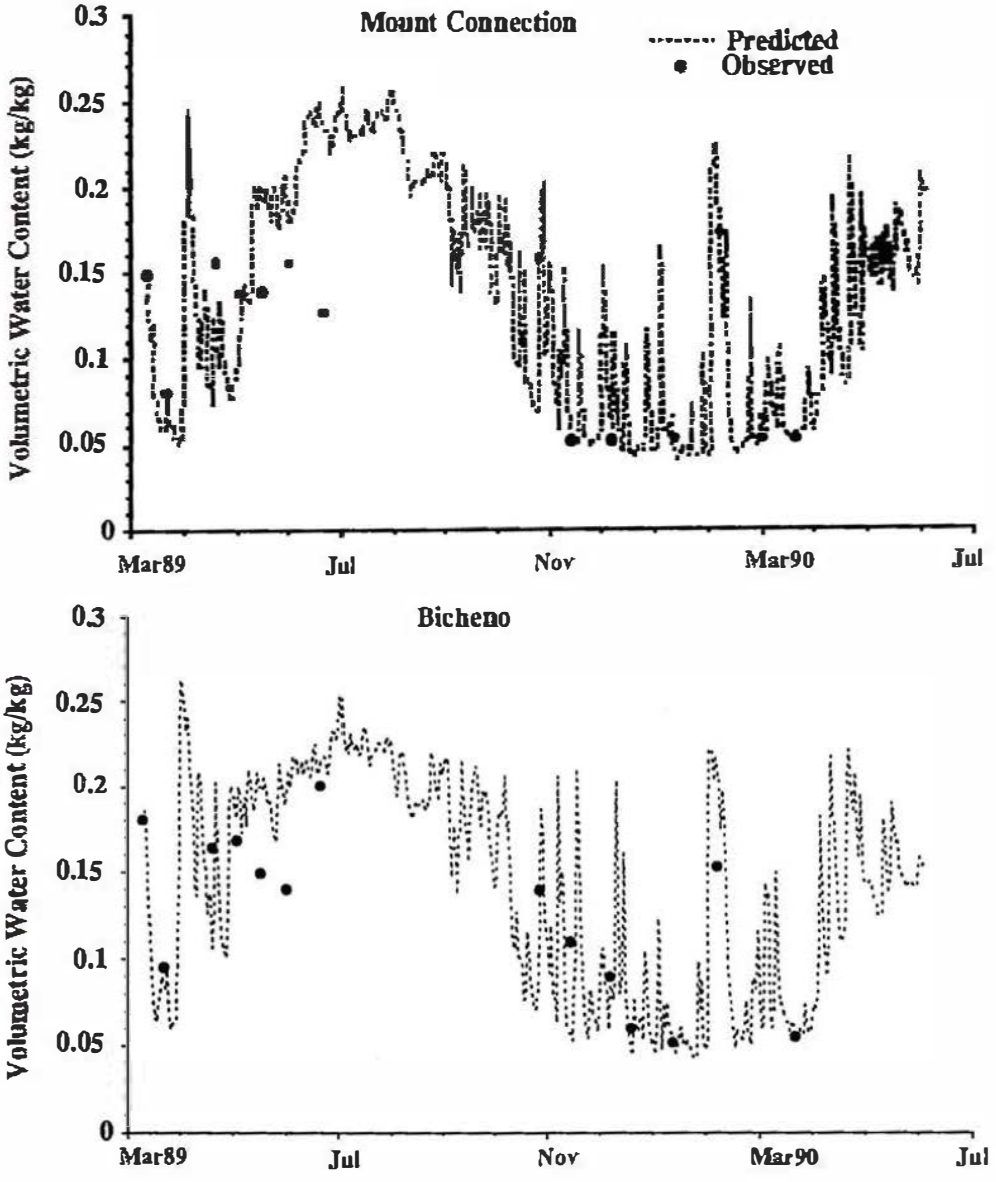

Fig. 8.1. Observed and predicted values of soil volumetric water content using the predictive model developed in Chapter 8. Volumetric water content was estimated from soil water potential using the formula, ψ=-exp(11.275-40.179.VVW), where ψ is the water potential in J/Kg.

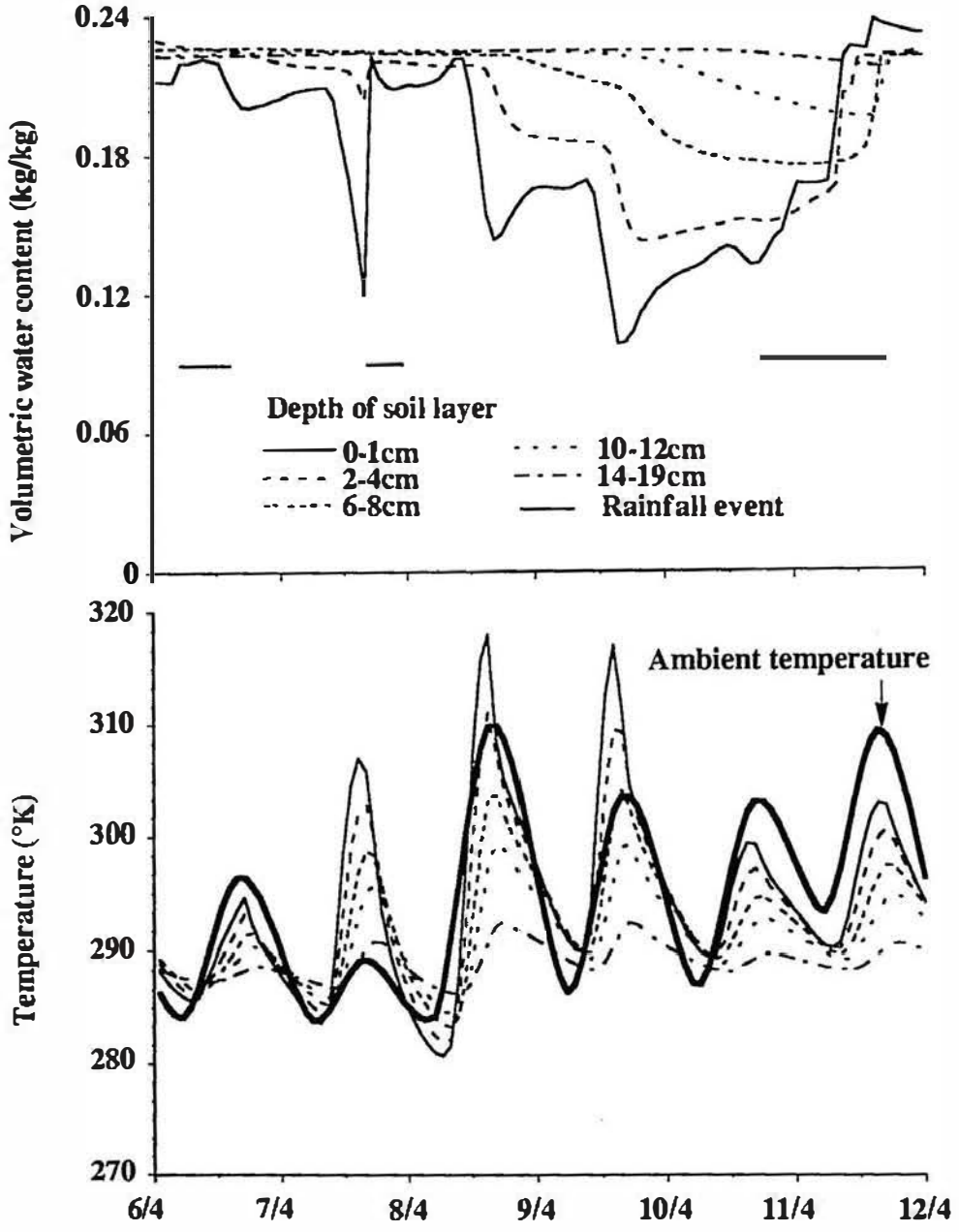

Fig. 8.2. Typical simulated patterns of diurnal volumetric water content and soil temperature for the period from midnight 5/4/89 to midnight 11/4/89 using the soil model developed in chapter 8.

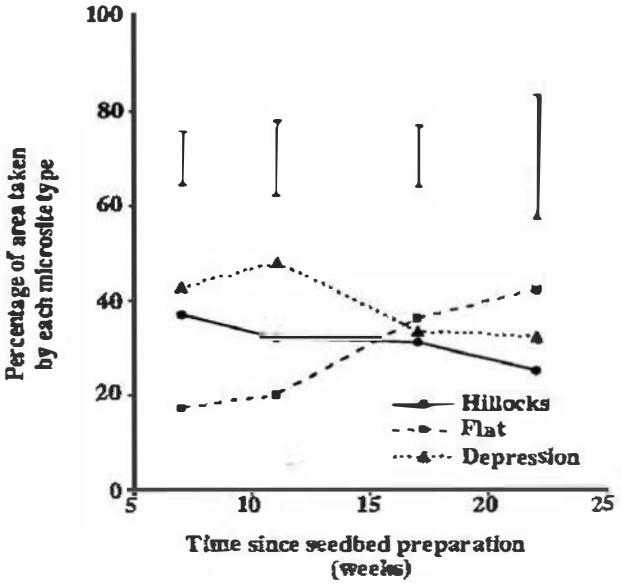

Fig. 8.3. Changes in seedbed characteristics with time. Error bars are the Tukey-Kramer least significant difference for multiple comparisons.

simulation modelling seedbeds were presumed on the basis of the above survey, to be divided into equal parts of the seedbed types. Seeds following sowing were considered to comprise three populations experiencing different ambient conditions according to microsite. The net response is the summation of the response of the three populations.

8.4 Attrition of seed from the seedbank

Total eucalypt emergence in the field is always less than the number of seeds sown. At most, 40% of the viable seeds are detected as seedlings (e.g. Cunningham 1960; Cremer 1962; Campbell and Bray 1987; also see Chapter 6). In Chapter 6 it was seen that the longer the delay between sowing and suitable conditions for emergence the smaller was the final proportion of seed detected as seedlings, although a delay over the warmer months appeared to be more detrimental than a delay over winter. A number of studies have shown that the depletion pattern of seeds from the soil is independent of seed age, indicating that age-independent losses due to factors such as disease and predation are more important than losses caused by senescence (Roberts and Dawkins 1967; Roberts and Feast 1973; Warnes and Andersen 1984). The severity of these factors is seasonally distributed. Seed harvesting, for example, was shown in Chapter 6 to be significantly higher during the warmer months of the year. This is, however, the only measure of the rate of seed loss gathered in the field studies for which germination prediction is planned. The seed bait approach is also noted for a number of severe limitations and at best gives a relative measure, rather than an absolute measure, of the rate of seed loss due to seed harvesting. Losses due to factors such as deep burial and fungal attack remain unquantified.

The removal of seed from the ground seed store was, therefore, modelled by presuming that a constant proportion of seed was removed each day, but that the amount could change from month to month. The monthly rates of seed removal were selected *a posteriori* to optimise the fit of observed emergence curves and predicted emergence curves. Nevertheless, these rates were selected to mimic the pattern of seed loss due as a result seed harvesting, probably the single most important cause of seed loss, though not necessarily to mimic the absolute levels. Seed harvesting was significantly higher at the Bicheno site and was significantly higher in the warmer months. This is reflected in the monthly seed attrition rates selected (Fig. 8.4). These rates were assumed to be constant, irrespective of the time since seed was sown.

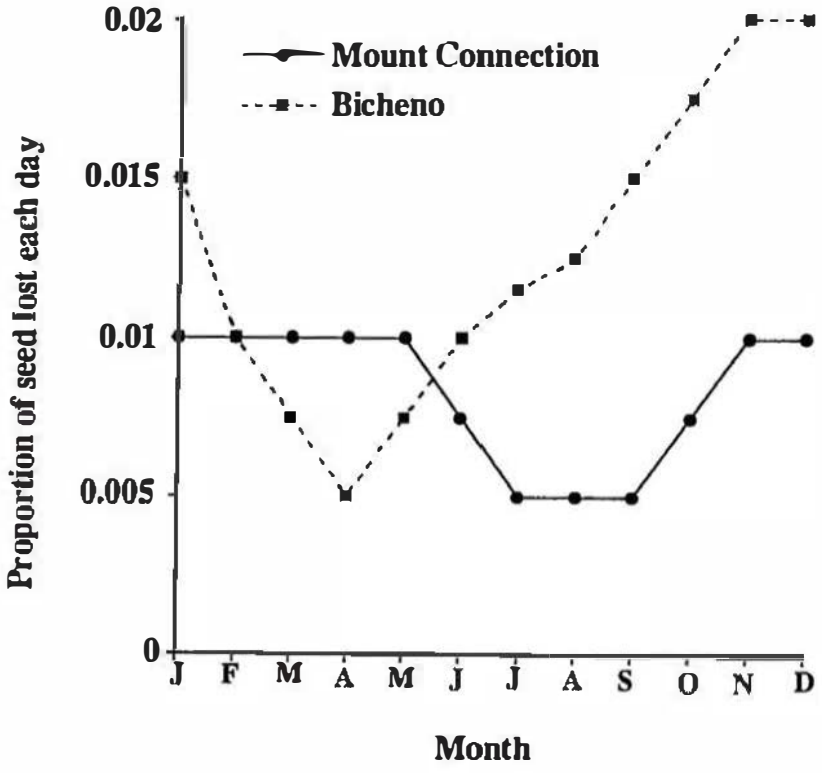

Fig. 8.4. Monthly rates of seed loss from the ground seed store at each experimental site used in the simulation of field emergence.

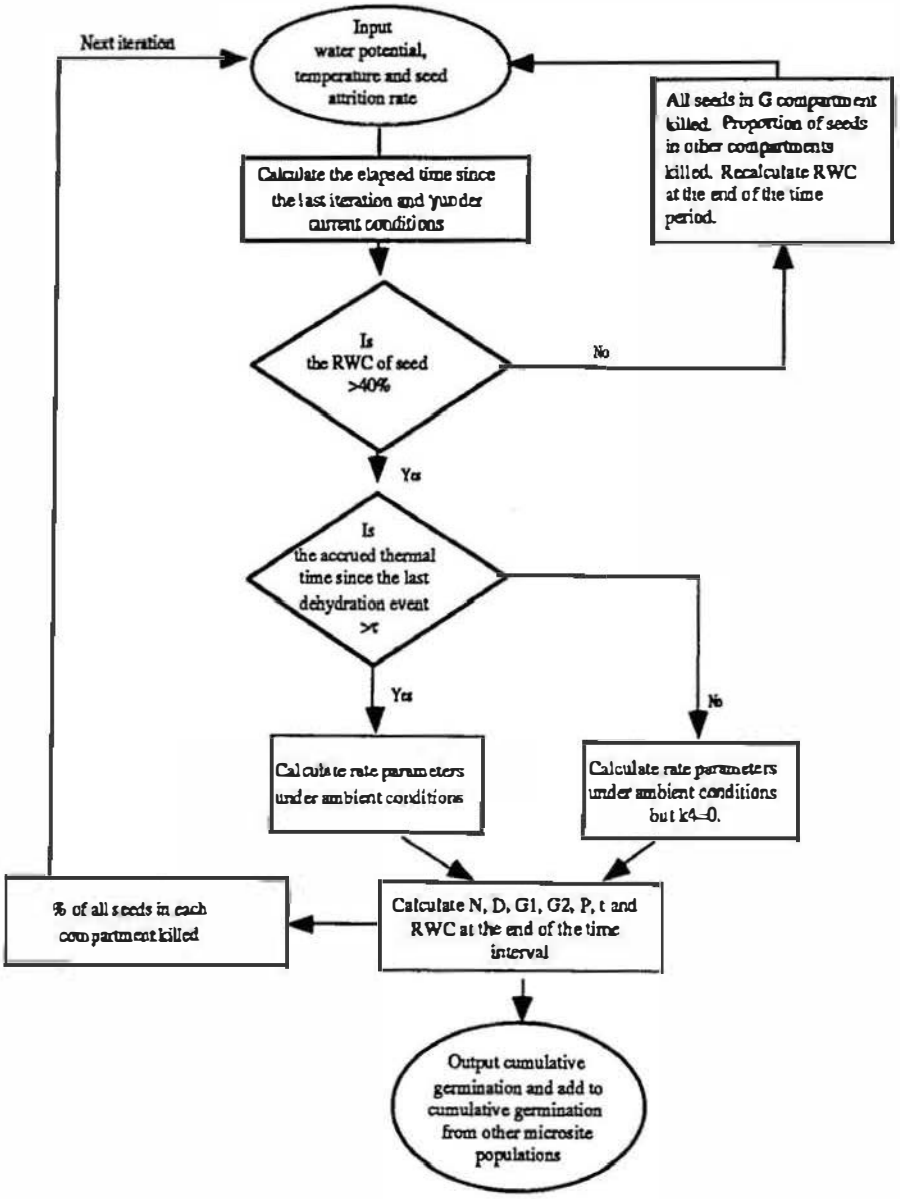

Fig. 8.5. Flow diagram of field emergence model for each microsite sub-population.

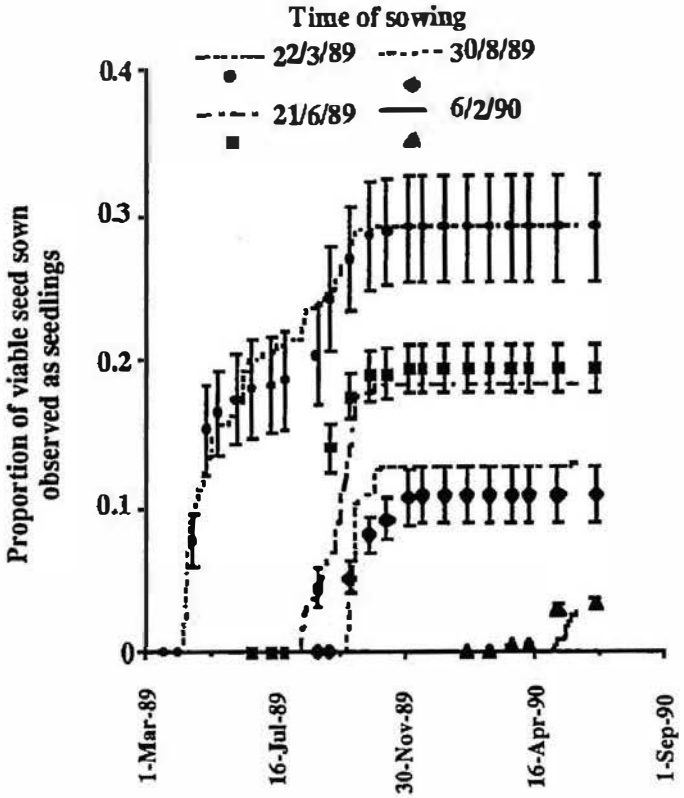

Fig. 8.6. Predicted and observed cumulative germination curves for sample sowing times at the Bicheno field experimental site. The error bars are the 95% confidence interval of the mean observed value (symbols) of all M36 delegatensis seed samples sown at that time.

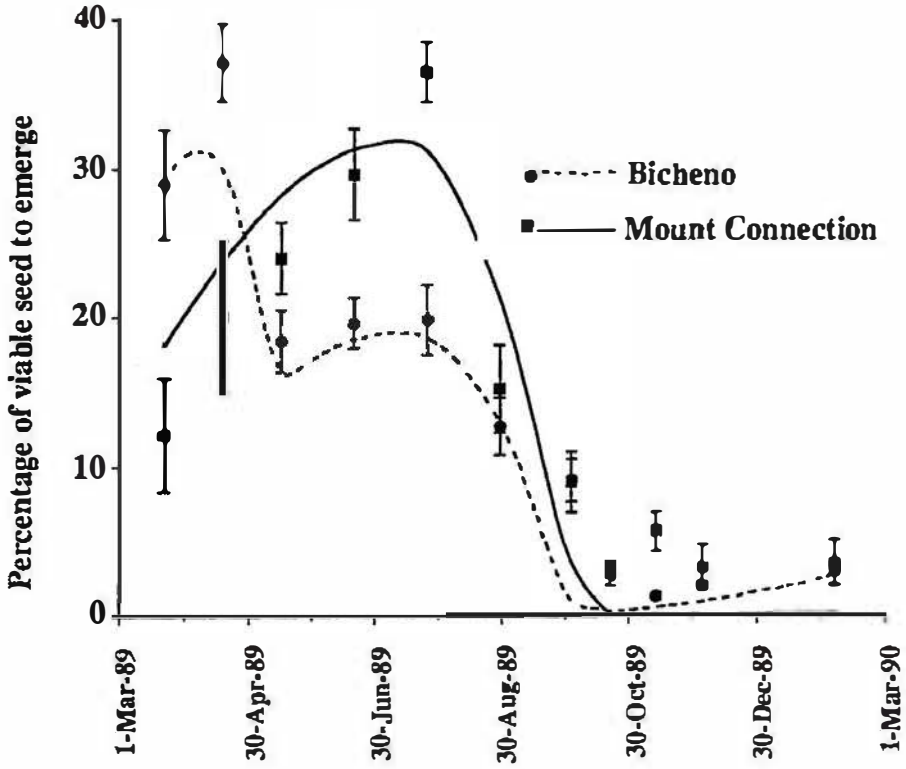

Fig. 8.7. The predicted (lines) and observed (symbols) percentage of viable seed to emerge following different times of sowing at the two experimental sites described in Chapter 6. Error bars are the 95% confidence interval of the mean observed value.

8.5 Predicting field emergence

These sub-models and the germination model from Chapter 7 were combined in the manner outlined in Fig. 8.5 and the model run for each time of sowing at both field sites with an hourly time step. Sample emergence curves from autumn, winter, spring and summer sowing times are given in Fig. 8.6. The predicted and actual cumulative germination from each time of sowing are given in Fig. 8.7. Because weather records do not begin until after the 10/3/89, the 2/3/89 time of sowing is not included.

The model predicts the relative germination performance of different times of sowing with reasonable accuracy. The model accurately indicates the comparative success of different sowing dates, and from a management and even research perspective this may be the most important criterion to be met. Predictions of germination from mid-spring to late summer sowings are consistently underestimated. Germination at this time is failing, in the model context, because seeds that initiate germination are being killed by dehydration in the dry periods between rainfall events and because of the high rate of seed removal from the system. This can be seen in Fig. 8.8a as the rapid increase in the proportion of dead seeds, relative to an autumn time of sowing, and in the step reduction in seeds in the G compartment. Following autumn times of sowing the dormant seed population, D, is shifted into the non-dormant pool, N, over winter and two flushes of germination occur. The non-dormant seeds sown in spring, however, are all killed prior to the onset of stratifying conditions, removing any opportunity for a second germination flush following the failure of the first. Because of the substantial delay prior to suitable conditions for germination following spring sowings, small errors in the estimation of the rate of mortality of seeds have a pronounced impact on the predicted cumulative emergence. Similarly the frequency of soil moisture fluctuations, magnify the impact of erroneous assumptions regarding the impact of seed dehydration and errors in the estimation of soil conditions. Detailed work investigating the fate of seeds once incorporated into the soil would clarify many of these issues. In this work the monthly rates of removal of seeds were selected to optimise the fit over the entire data set, consistent with the pattern of seed harvesting detected at each site. Assumptions regarding an identical mortality rate for seeds in all stages of germination, on all microsite types and of all ages are almost certainly an over simplification. Seeds incorporated into the soil matrix, for example, may well be less vulnerable to predation.

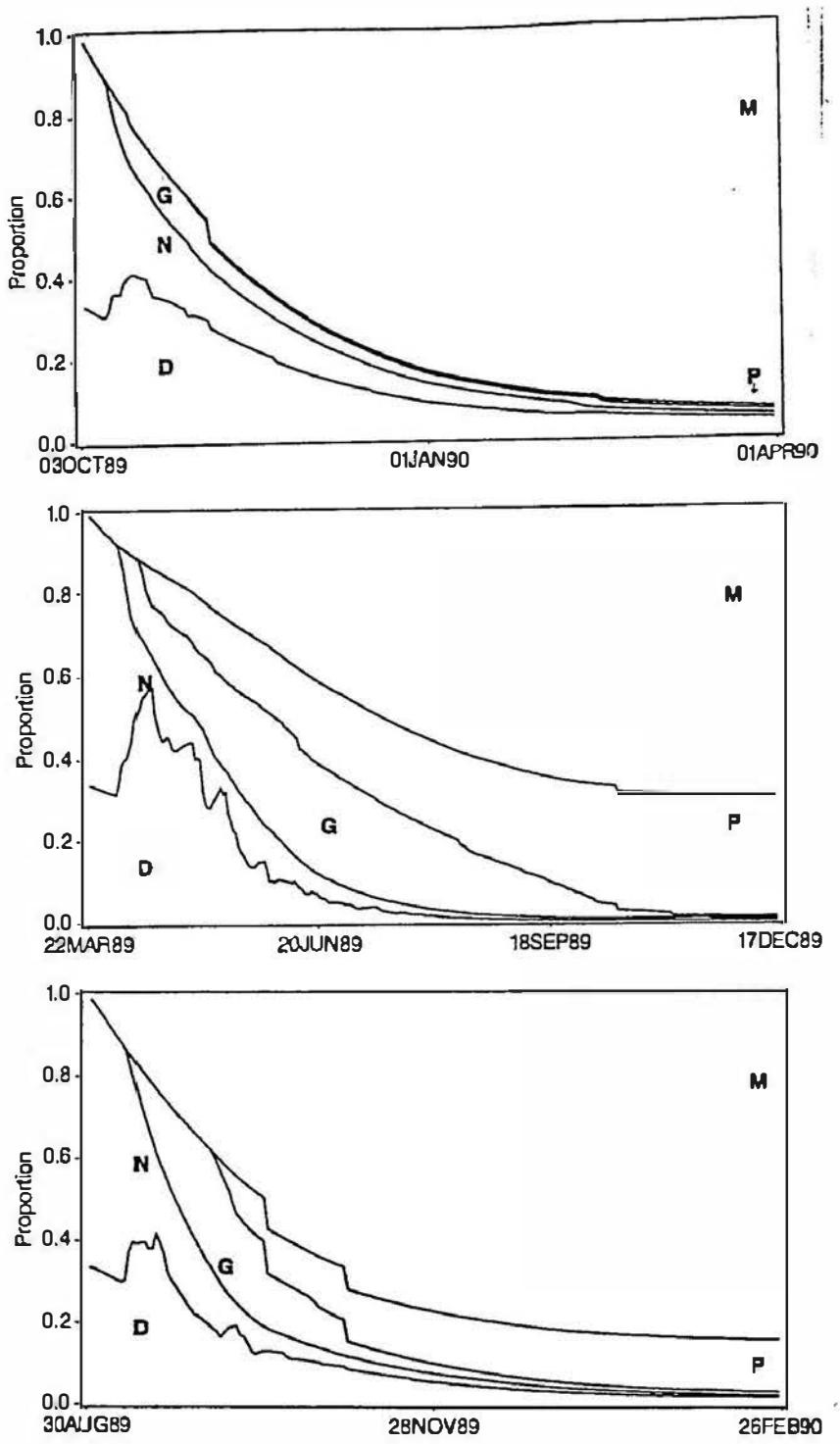

Fig. 8.8. Proportion of seeds in the different model compartments over time following sowing in a) mid-spring (3/10/89), b) autumn (22/3/89) and c) early spring (30/8/89). The conditions used in the simulation are the conditions that occurred at the Bicheno experimental site during 1989-1990. **M**=that proportion of seeds removed from the system by senescence, decay, predation etc., **P**=successfully germinated seeds, **G**= seeds ready to germinate and which are irreversibly committed to the emergence process, **N**=non-dormant seeds at a preliminary stage of germination which may be dehydrated without damage, and **D**=seeds that are dormant.

The field predictions provide confirmation of the seed germination sub-model developed in Chapter 7. The initiation of germination flushes in autumn and spring, and the rates of emergence are predicted accurately (Fig. 8.6). The germination sub-model performs well under conditions of fluctuating temperature and moisture. The field predictions also indicate that soil moisture is being adequately represented by the soil moisture sub-model developed earlier in this chapter. The cessation of germination at the end of spring and the onset at the end of autumn 1989, in particular, and to a lesser extent autumn 1990, are predicted accurately.

The prediction of germination flushes by the field emergence model indicates that the germination model developed in the previous chapter is plausible. The accurate prediction of cumulative field emergence in this chapter, however, does not necessarily validate the field emergence model since the selection of parameter values to describe the rate of attrition of seed from the ground seedbank was made to optimise the fit predicted to observed germination curves, and is not based on observation of processes nor tested with an independent data set. The hypotheses regarding the rate of seed loss are easily testable, and given the significance of these to the regeneration outcomes, ought to provide the subject of future research.

The modelling process can be used to test some assumptions regarding the dynamics of the seedbank processes that are affecting the pattern and timing of seed emergence. Grose (1957a) for example, explained the relatively lower cumulative emergence of late spring than autumn sowings as the result of the loss of the death of the dormant seed fraction before the onset of stratifying conditions. The modelling process (Fig. 8.8a) is consistent with this hypothesis. The bimodal distribution of emergence from autumn sowings and the unimodal distribution of emergence times from winter and early spring times of sowing observed in Chapter 6 and many other eucalypt germination studies (e.g. Cremer 1962; Fagg 1981) has been explained by stratification of winter and early spring sown seed almost immediately following sowing, compared to the time delay prior to stratifying conditions following autumn sowings (compare Fig. 8.8b and 8.8c).

It has been widely presumed that very little viable seed remains more than 12 months after the time of sowing (see Lockett 1991). This is supported by the model output (Fig. 8.8) which indicates that within six months of both spring and autumn sowing times the combination of germination and seed mortality will

have removed over 95% of the viable seed originally sown. Twelve months following sowing virtually no viable seed is predicted to remain in the soil.

It is commonly assumed that the superior emergence observed on recently prepared seedbeds is associated with the higher proportion of safe sites (Lockett 1991). In Chapter 4 it was suggested that 'safe sites' had a temporal as well as a spatial distribution. That is, the safety of a particular microsite type is dependent upon seasonally-distributed patterns of soil moisture and temperature. These hypotheses can be tested in the model context by examining the proportion of seeds emerging on different model microsite types, and by examining the effect on cumulative emergence of varying the proportion of microsites.

While the model suggests that germination is always higher on favourable microsites (depressions), these differences are greatest for the early autumn sowing time, 22/3/89, when emergence is concentrated in the intermittently moist months of April and May, and for the early spring time of sowing when germination is concentrated in the mid to late spring under conditions of drying soil (Fig. 8.9). Differences from late autumn and winter sowing when germination is concentrated in the moist conditions of early spring are less pronounced. Late spring and summer sowing times result in poor emergence on all microsite types, however some emergence, albeit only between one and two per cent, occurs on the most favourable microsite irrespective of sowing date. As was found in Chapter 4 it appears that 'safe sites' are demographically more important in variable or adverse environments.

If the proportion of safe sites, depressions in this case, is varied from 0% to 100% of the seedbed area for a particular sowing date, the effect of the time elapsed between seedbed preparation and sowing can be examined, albeit in an exaggerated manner. Immediately following seedbed preparation by either burning or mechanical disturbance most seed becomes incorporated into the uppermost soil layers, but as time passes (see Fig. 8.3) the number of favourable microsites declines and seeds germinate in a harsher microenvironment. Seeds sown on the 30/8/89 onto a seedbed with no safe sites would have resulted in 8% of the viable seeds emerging, compared with 19% on a seedbed in which all sites are safe (Fig. 8.10). More significantly, following a dry spell at the end of October only seeds on safe sites survived. If the recently germinated seedlings had also been killed by this dry spell, regeneration was dependent upon the reservoir of seeds on safe sites. The importance of 'safe' sites for the storage of seeds during adverse conditions was also observed in the glasshouse experiments

in chapter 4. Eucalypt seed shed, particularly following wildfire when it can be as high as 2 600 000 seeds ha^{-1}, or somewhere in the vicinity of 25 000 seeds per tree, (Campbell *et al.* 1990) is high by comparison to many trees (Gashwiler 1967; Harper and White 1974; Sivertown 1982). Unlike many trees which seed more abundantly (e.g. >1 000 000 per tree for *Salix lasiolepis,* Sacchi and Price 1992) dispersal is confined to a short distance from the tree, with a single tree capable of seeding an area of radius equivalent to its height (Cunningham 1960; Cremer 1966; Cremer 1977). Most of the available microsites within this area are, therefore, saturated with seed. By ensuring favourable microsites are occupied, this response may mitigate the effects of within season variability in factors such as soil moisture which define the regeneration niche.

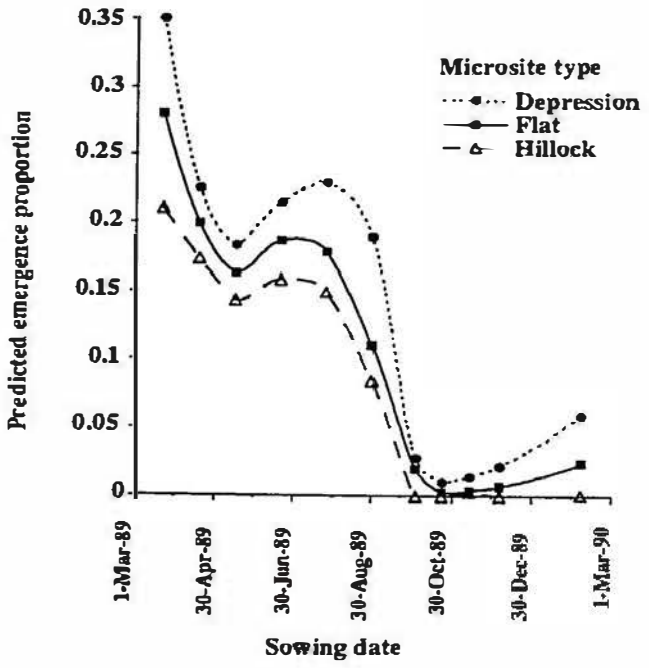

Fig. 8.9. Predicted proportion of seeds to emerge on different microsites from sowings made on different dates.

Sowings of the disparate provenances introduced in Chapter 2 can be simulated to test hypotheses about dormancy and fitness raised in Chapter 6. It was assumed from data in Chapter 2 that L17 provenance was non-dormant, and that full germination could occur at temperatures up to 22°C. The M50 provenance was assumed to possess seed that was 70% dormant, and that dormancy relief by stratification occurred at only 25% the rate of the M36 provenance. It can be seen (Fig. 8.11) that given the same weather sequence, a wet autumn followed by a cold winter, the provenances give substantially different germination patterns. Because seed of the less dormant provenances germinate more promptly, losses are fewer and cumulative germination higher. The frequency of very severe frosts (terrestrial minimum<-8°C) at the Lake Leake meteorological station, near to the Mount Connection experimental site, is given in Table. 8.2. These frosts will kill all seedlings less than six months old, and probably most considerably older. By calculating the respective conditional probabilities the expected number of seedlings for each provenance surviving at the start of the December following "sowing" can be determined. Seedlings germinating early have a reduced probability of surviving since they will be exposed potentially to more frosts. Approximately 3% of the L17 provenance can be expected to be alive as seedlings in December, 7% of the M36 seed and 13.3% of the M50 seed. However it can be assumed that seedlings germinating early will be in a better competitive position relative to seedlings germinating later. Using the figures of Campbell and Bray (1987) as a guide it will be assumed that 70% of surviving seedlings from March germination times will be in a dominant competitive position by December, with the dominance of later times of germination reduced by 5% a month after this. Using these figures, 1.5% of the L17 seeds will result in dominant seedlings, 3% of the M36 seed and 5% of the M50 seed. If the findings in Chapter 6 that on particularly cold and frosty sites, spring germinants have an competitive advantage over autumn germinants is correct, this difference in "fitness" becomes even more marked. If the killing frost frequency is reduced by two thirds (Table 8.2), to give a temperature regime more compatible with the Bicheno experimental site, the figures for cumulative germination become: 15%, 15% and 17% and for dominance 10%, 8% and 7%. Consideration of ontogeny and drought tolerance will change these figures slightly more in favour of autumn emergence.

This modelling, therefore, suggests that the particular pattern of emergence, determined largely by the dormancy profile of the seed population, which imparts greatest fitness is a "trade-off" between hazard avoidance and the ability of subsequent seedlings to maintain a competitive position relative to seedlings

arising from earlier germination times. On frosty sites, spring germination which increases the chances of survival, may also enhance growth (Fig. 6.15), providing a double incentive for delayed germination. These modelling assumptions are supported by the demographic data in Chapter 6. At the frosty Mount Connection site, late winter and early spring emergents had greater mean life expectancies than did mid- to late-autumn emergants, although early-autumn emergants were also favourable (Fig 6.12). At the more benign Bicheno site, there was very little distinction between the safety of particular emergence times and clearly establishment of a competitive advantage by early growth is paramount, and not suprisingly the dormancy profile of seed collected from near this site (Fig. 3.4) indicates that trees produce seed with little inherent dormancy.

Table 8.2. Probabilities used to calculate outcomes of sowing differing provenances at two climatically dissimilar sites.

	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Probability of killing frost during the month									
MC	0.07	0.21	0.36	0.57	0.5	0.57	0.07	0.07	0.07
Bi	0.00	0.07	0.11	0.19	0.16	0.19	0.02	0.02	0.02
Conditional probability of seedlings germinating in that month being alive in the following December									
MC	0.034	0.037	0.047	0.074	0.173	0.346	0.80	0.86	0.93
Bi	0.42	0.42	0.46	0.51	0.64	0.76	0.94	0.96	0.98
Proportion of surviving seedlings that will be in a dominant competitive position									
	0.7	0.65	0.6	0.55	0.5	0.45	0.4	0.35	0.3

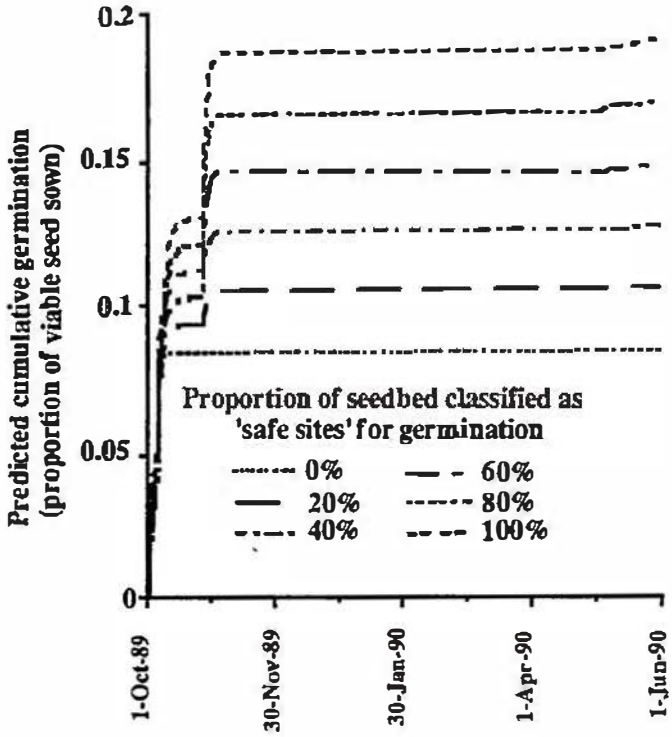

Fig. 8.10. The effect on predicted emergence of changing the proportion of safe sites from 0% to 100% for sowings made 30/8/1989 at the Bicheno field experimental site.

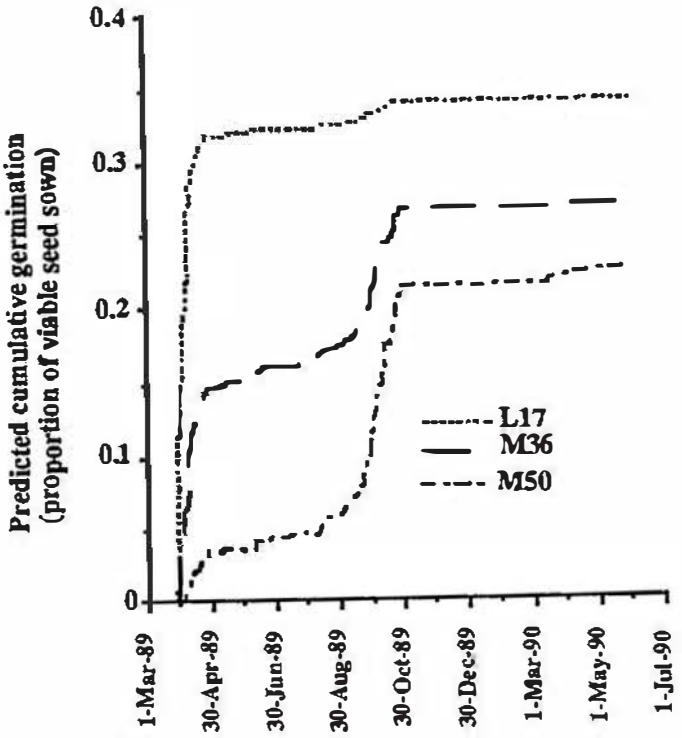

Fig. 8.11. Predicted patterns of emergence of different seedlots (see Chapter 2) using weather conditions simulating a wet autumn and winter at the Mount Connection field experimental site.

Chapter 9 : Conclusions

9.1 Defining the regeneration niche of *Eucalyptus delegatensis*

The identification of the optimum time at which to sow *Eucalyptus delegatensis* seed during reafforestation activities, necessarily, involves the definition of the regeneration niche. Grubb (1977) defines the regeneration niche of a plant as "... an expression of the requirements for a high chance of success in the replacement of one mature individual by a new mature individual of the next generation ...". Processes of seed set and dispersal, seed survival and germination and seedling growth are all involved. In artificial regeneration terms the regeneration niche begins following sowing or seedfall induction; the seed-set and seed-dispersal components of the regeneration niche have been circumvented.

It may take *E. delegatensis* 15 years or more to reach sexual maturity, and strictly speaking, the regeneration niche, in Grubb's (1977) terms, needs to be considered over this time span. However, not all "environmental stages" are necessarily of equal relevance in a demographic sense (Sarukhan *et al.* 1984). For *E. delegatensis*, as for many other species (see Bazzaz *et al.* 1982 for examples), the time of germination and establishment is demographically the most crucial, and determines whether the species retains site occupancy and which individuals of the species will reach sexual maturity. Fewer than 1 in 1000 dispersed seeds will result in a sexually mature individual (in fact if all the propagules produced between regeneration events are considered this may be closer to 1 in 10^7 seeds!). Most of the propagules, both seeds and seedlings, are lost in the first year or two following dispersal. Only 1 000, or even fewer, seedlings per hectare may survive at age two from the 100 000 seeds per hectare originally dispersed. In conventional demographic terms the survivorship curve of the species follows the Deevey type III survivorship curve (Deevey 1947) with a very high rate of juvenile mortality and a low adult mortality rate (although adult survival may be truncated by a cataclysmic wildfire). Clearly, the crucial factors that influence the regeneration niche and establishment of *E. delegatensis* are those that influence population numbers in the first 12 to 24 months following seed dispersal. Subsequently, the ability of the species to retain site occupancy may be determined by infrequent climatic events such as droughts or frosts with a low return period (Davidson and Reid 1985; 1989).

9.1.1 Seed Survival

In this work, only a proportion, up to a maximum of 40%, of the *E. delegatensis* seed sown in the field was detected as seedlings (Fig. 6.6). Seed dispersed at unfavourable times for seed survival and germination resulted in a much lower conversion rate than this, with only 0.11% of seed sown detected as seedlings. These figures are similar to those found in other studies of eucalypts (Cremer 1962; Fagg 1981); the upper figure is respectable relative to other species, while the lower figure is particularly low (see Symonides 1988 for comparison to studies of annual plants). Understanding what determines the fate of these seeds is central to defining the regeneration niche.

Experimentation indicates that during the warmer months of the year, seed harvesting may account for much of the missing seed (Fig. 6.8). Glasshouse work and modelling indicate that during the drier months losses due to dehydration of partially germinated seeds may also contribute significantly to seed loss (Fig. 4.3 & Fig. 8.8). Other work has indicated that decay may be another important seed-loss factor (Neumann and Kassaby 1986). Seed dispersed when conditions are unfavourable for germination, and consequently stored in the soil for a prolonged period of time, can suffer substantial losses. For example, seed dispersed late in spring does not encounter substantial periods during which conditions are suitable for germination prior to the autumn of the following year (Fig. 6.5). Even with the arrival of warm moist conditions in early autumn a fraction of the surviving seed requires the cooler conditions of late autumn and winter to break dormancy and, hence, will not be ready to germinate, if it survives, until possibly the next spring. Losses in the intervening period appear to be high since, in this study, very little germination resulted from such sowing times (Fig. 6.6).

9.1.2 Germination

Both temperature and moisture appear to be key components in the control of germination (Fig. 2.5 & 2.9). The regeneration niche of stratified and non-stratified M36 provenance *E. delegatensis* seed, reduced to the two dimensions of temperature and water potential, is indicated in Fig. 9.1a&b. However, because of seed losses with time due to factors such as seed harvesting and decay, this regeneration niche is confined, in practice, to those temperatures at which germination progresses at a reasonable rate (Fig. 9.1c). It was found in the field that if temperatures failed to rise above 10°C for a significant proportion of the day very little emergence was detected. This was confirmed in controlled-

environment experiments that showed that the rate of germination at temperatures of 12.5°C or below was very slow (Fig. 2.2). A similar threshold of soil waterpotential of -0.5 MPa was manifest in both controlled-environment (Fig. 2.10) and field situations.

In addition to these two dimensions, a third dimension of time is required to explain germination performance. The timing of temperature and moisture conditions, as well as the absolute level, is important, and hence, a seed's response to current conditions is determined, in part, by the preceding . For example, seed subjected to prolonged cool moist stratification is comparatively insensitive to the ambient temperature, whereas seed without prior stratification has a well defined temperature optimum for germination (Fig. 2.5). Furthermore, dehydration of seeds after a certain stage of the germination process results in the death of a proportion of seeds. Immediately following the commencement of imbibition, seeds appear resistant to the effects of dehydration, but as the processes of germination, seed coat rupture and radical extension progress, resistance decreases (Fig. 2.18). Seeds subjected to water stress or to low temperatures advance more slowly toward germination and hence require a longer period of suitable conditions. In these instances, temporal variation in conditions is more critical. There is, therefore, an interaction between the axes defining the environmental constraints of germination and the time axis in determining the ultimate germination outcome.

As well as being temporally variable the regeneration niche is spatially discontinuous. Micro-topographical changes strongly influence the distribution of seedlings (Fig. 4.2). The comparative 'safety' and suitability of a particular microsite for germination varies with climatic conditions (Fig. 4.3). Under favourable conditions the effect of microsites is comparatively slight, however, if conditions are marginal for germination, or seeds remain in the soil for long periods of time prior to germination, microsite effects can become pronounced. Consequently, the use of the laboratory studies to derive the mean response of seeds is not adequate for the prediction of the timing or location of field emergence without consideration of the spatial and temporal heterogeneity of soil conditions. The regeneration niche, therefore, although delimited by soil water and temperature, is dynamic in space and time.

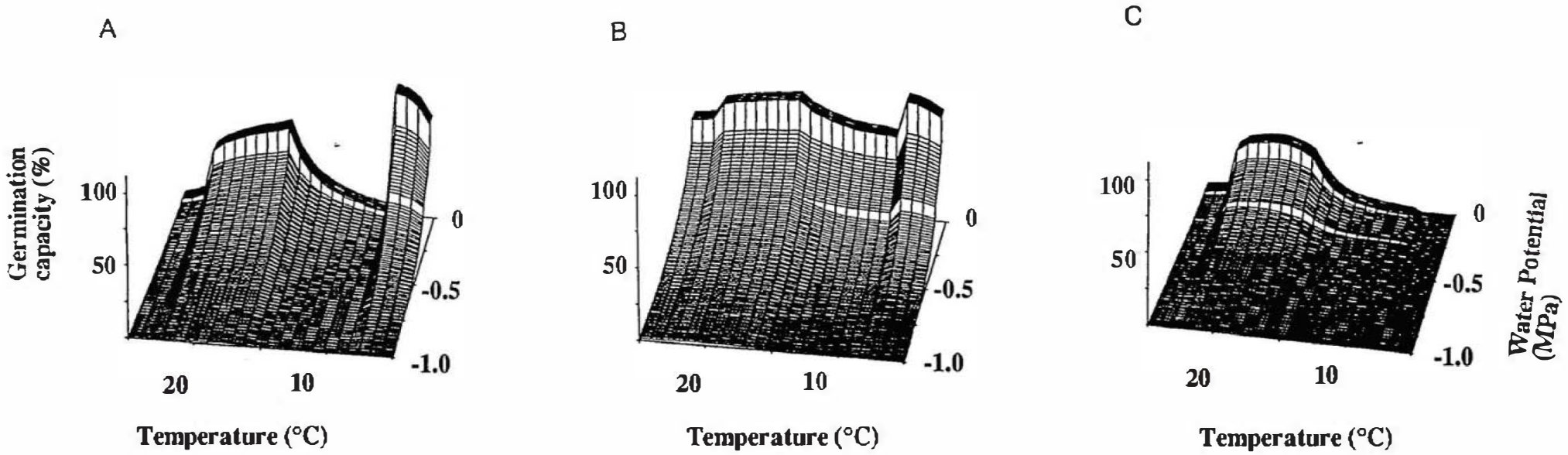

Fig.9.1. The germination niche of *E. delegatensis* defined by temperature and water potential of: A) non stratified seed B) stratified seed, and C) the area of response where the germination rate is sufficiently rapid to negate the effects of seed destroying factors.

9.1.3. Seedling survival

Frost heave and frost tissue damage and drought were the principal seedling mortality factors acting in the field study. Insect defoliation was a significant mortality factor but its effects were minor compared to the effects of frost and drought. The effects of mammalian browsing were excluded from this study by fencing. Seedling frost and drought hardiness were found in glasshouse and laboratory studies to be related to seedling developmental stage (Fig. 5.1). In the field seedlings germinating late in autumn and late in spring had reduced survival chances relative to early emergents. This was clearly related to seedling susceptibility to frost and drought respectively. Mortality risk was, however, not always influenced by seedling age and one severe frost in late autumn killed a high proportion of seedlings of all ages (including nearby saplings) at the Mount Connection site (Fig.6.10).

The extent to which a seedling must develop to be safe from the average vagaries of weather is unclear, but it appears that once seedlings have developed two fully-expanded leaf pairs they are considerably more robust than are seedlings possessing only cotyledons (Fig. 4.5 & 5.1). The gain in frost and drought tolerance as seedlings develop from the two-leaf to the four-leaf stage and beyond is slight by comparison. There was not a good correlation between age and developmental stage in the field, even within a site. Growth rates were slow, and twelve months after sowing, seedlings from the one emergence time ranged in height from 1 cm tall seedlings with only two leaves to 10 cm tall seedlings with many leaf nodes. Nevertheless, it is probably reasonable to add to our regeneration niche criteria the requirements that, following emergence, seedlings need a period of at least two and possibly three months without severe frosts or drought. The actual intensity of these stresses required to cause damage in the field have not been precisely defined in this study. Laboratory studies indicated that temperatures below -2°C would kill most hardened cotyledon stage seedlings. In the field, however, widespread tissue damage was only detected following a frost of below -7°C, but frost heave of small seedlings was prevalent when temperatures fell to below -2°C. Lesser degrees of tissue damage were recorded among recently emerged cotyldedons following milder frosts. Drought related death was found in glasshouse studies to be influenced by microsite effects as well as age. Nevertheless, substantial numbers of seedlings died in the field when the water potential of the surface soil fell below -1.5 MPa (a volumetric water content of 0.1 kg/kg).

The shape of survivorship curves was variable between sites and times of emergence (see Appendix 3). As has been found in other studies (e.g. Symonides 1974; Zeide 1978; Klemnow and Raynal 1981) the shape of survivorship curves can not be considered to be characteristic for a given species. While in many cases, the general form of the survivorship curve could be satisfactorily approximated by the negative exponential function, a severe frost at one of the experimental sites from which demographic data was collected meant that allowance for a disruption to these curves in the second autumn of observation was necessary (Fig. 6.11). Seedlings on the harsher of the sites studied were characterised by high rates of mortality with some evidence of seasonality in the severity of mortality hazard, whereas seedlings growing on a comparatively mild site were characterised by low rates of mortality, which appeared to decrease markedly after the first weeks of life, and by no clearly identifiable high risk period with the possible exception of a slight increase in hazard for very young seedlings during winter.

9.1.4 Regeneration strategy

Seed of *E. delegatensis* has only a short duration of soil residence. Very little seed germinates more than 12 months after dispersal (Fig. 6.5). Transient seedbanks of this kind (that is seedbanks where there is no carry over from one year to the next) are characteristic of habitats in which there is a high annual probability of successful reproduction (Symonides 1988). Such seedbanks may include both dormant and non-dormant seeds if the optimum time for germination has a high degree of intra-year variability (e.g. Arthur *et al.* 1973; Klemnow and Raynal 1981). There are very few years in which both the spring and autumn are unsuitable for the germination and establishment of *E. delegatensis* within its natural range, but, nevertheless, considerable year to year variability in conditions occur (Battaglia 1990; see Chapter 6). The frequency of frost and drought vary substantially across this range. Just as long-term seedbanks can be regarded as a form of 'bet hedging' with regard to inter-year variability, periodic germination through the year, or over some part of the year, in a variable climate increases the likelihood that at least some seeds will germinate during favourable conditions and survive to reach sexual maturity and reduces the impact of intra-year variation (Cohen 1968; Symonides 1988; Venable 1989).

It has been suggested that the proportion of seed in temperate climates that is dormant will depend upon the relative probability of spring and autumn germinants surviving (Venable 1989). This assumption is supported in this study

by the comparative dormancy of seedlots drawn from provenances with varying severity of autumn and winter dormancy (Fig. 2.6), and by simulation modelling which indicated that a high proportion of dormant seed increased the long term chance of successful establishment on a very frosty site compared with a mild site (Fig. 8.11 and Table 8.2). This trend towards greater dormancy increasing fitness on frosty sites appears to be reinforced by the observation in this study, and other eucalypt (Cunningham 1960, Cremer 1962; Fagg 1981) and non-eucalypt (Miller 1987) studies, that seedlings that over-winter on frosty sites, or are subjected to frosts early in the growing season, grow slowly relative to spring, or late season, emergents on the same site (Fig. 6.15). On these very frost-prone sites there is a clear advantage in a high proportion of seed germinating in spring, and it can only be the risk of a dry spring resulting in the death of all spring emergents that prevents all seed being highly dormant.

There appears to be a similar within-tree variability in the ability of seeds to germinate at different levels of water stress. As with dormancy, this acts to disperse germination in time, and ensures that not all seeds germinate, for example, following short, wet spells in spring or summer. Variation in this trait and the differential levels of dormancy in the seeds from the one tree, indicate that because of year to year variability in the optimum time of emergence there is no one distinct optimum time of emergence, but rather optimum fitness involves ensuring a spread of emergence times and a degree of 'bet hedging'. Different sites are characterised by a mean germination characteristic response that is related to the average conditions (i.e., trees from colder sites produce seed that has a higher proportion of dormancy, and trees from drier sites produce a higher proportion of seeds that can germinate under water stress), but all sites are characterised by a substantial variation around this mean response. Thus, there appears to be directional selection towards a mean response at the provenance level, but between year variability in conditions, and possibly local scale spatial heterogeneity in soil conditions acts in a disruptive manner and ensures that a high degree of variability in response is maintained both within the population and within the seed from the one tree.

The seed rain from eucalypts, including *E. delegatensis*, is intense with literally millions of seeds falling per hectare. This may be another response to ensure regeneration success in the face of environmental variability. By ensuring that some seed falls onto favourable microsites the effect of within-season variability in factors such as soil moisture may be mitigated. 'Safe sites' may provide

important refuges for seed and prevent an adverse weather event removing all potential propagules (Fig. 8.10).

E. delegatensis is geographically widespread in Tasmania It occurs as a member of dry sclerophyll communities and as a rainforest emergent, and in communities intermediate between the two. It occurs on sites with few, and mild, frosts as well as on some of the more frost prone sites in the state. The species displays germination characteristics which minimise the chance of germination at times when the probability of successful establishment is low. Furthermore, the species displays substantial within and between-provenance variability in germination characteristics, including a seed dormancy proportion that ranges from at least 70% of the total seed fraction to none of the seed. In addition, seed germinates and survives over a wide range of temperatures and soil moisture conditions. Many environments offer the appropriate combination of conditions at some time during the year. A regeneration strategy that disperses germination in time, as well as prolific seed shed, ensures that *E. delegatensis* is able to exploit these germination windows when they arise. This opportunistic approach to securing a germination opportunity, combined with the frost hardiness of seedlings and adults, undoubtedly accounts in part for the geographic success of the species.

Modelling of the fundamental and/or realised niches of species has been used extensively to explain patterns of plant distribution and to predict occurrence (e.g. Austin *et al.* 1983, 1984; Busby 1986, 1988; Margules *et al.* 1987; Nichols 1989; Yee and Mitchell 1991). The regeneration niche is an important component of the fundamental niche of a species, and it has been suggested that species diversity has more to do with requirements for regeneration than with partitioning of the habitat niche of the adult (Grubb 1977). The physiological environment to which a tree is subjected at any stage in its life-cycle will affect the continued survival of the individual. To explain the distribution of a species it is important, therefore, to analyse the habitat niche for different stages of long-lived individuals. Most analyses of the species niche in eucalypts has focussed on adults or established seedlings, thereby overlooking the important earlier stages of the life-cycle. Resources that are fine grained to saplings or adult trees may be experienced as coarse grained by seeds and seedlings of the same species in the same environment. Harper (1977) and Grubb (1977), among others, have suggested that the events that determine the different fates of individual plants frequently occur during the period of the life cycle encompassing seed dispersal, germination and seedling establishment. If so, the nature of the environment immediately surrounding a seedling and its effect on that seedling will be highly

significant in determining future plant community composition. Differences in establishment requirements and strategies may result in particular species pre-empting local access to resources. Consequently, local scale distribution of species may be determined, in part, early in stand development by environmental differences in the regeneration niche and the role of interactive competition between adult plants in determining distribution reduced in significance. In frequent events with a low return period, such as the frost discussed in Chapter 6, may also play a significant role in species composition (see also Davidson and Reid 1985; 1989).

The regeneration niche, for *E. delegatensis* at least, is relatively easily defined using field-based demographic studies and laboratory and glasshouse experimentation. Definition of the regeneration niche of a species may be the most easily quantified component of a species' niche. Incorporating information on the regeneration niche, such as the probability of dry spells during the normal time of seed germination, may allow a more precise definition of the fundamental niche of species. Furthermore, it has been suggested that differentiation in the regeneration niche allows poor competitors to establish episodically when the dominance of the more competitive species is disrupted (Pickett 1980). It is likely that inclusion of some measure of the regeneration niche into predictive models will result in a more precise definition of a species distribution.

The detailed information about the regeneration niche developed in this study provided a sound basis for seed germination modelling, and ultimately successful prediction of the timing of field emergence of *E. delegatensis*. While models have value for quantitative prediction, their most significant value may be as heuristic aids to the understanding of the performance of the systems that they attempt to describe (Charles-Edwards 1982). The modelling process in this work has allowed the investigation of the impact of factors such as the extent of seed dormancy and the influence of heterogeneity of soil conditions on population dynamics. It has also supported a number of casual field observations regarding the residence period of seed in the soil and concerning the origins of different patterns of emergence from different sowing times. This information is of as much management significance, and of more general ecological interest, than is the precise prediction of sowing-time outcomes.

9.2 Management implications arising from this work

This work has stopped short of predicting the optimum time to sow seed in the field. The easiest way of obtaining these predictions lies in the development of models to simulate typical weather sequences (or drawing random years from the weather record, if this exists) for a network of sites across the geographic area of interest. These can subsequently be used to predict the outcomes from sowing dates using the field emergence model developed in Chapter 8. The accumulated outcomes from given sowing times can then be use to select the sowing date that best meets the selection criterion, whether this be to maximise average yield or minimise the probability of failure. Models that generate typical weather streams and their outputs are used as inputs for crop simulation models (e.g. Guenni *et al.* 1991; White and Russell 1991). There is scope for future work to apply these techniques to the problem of assessing the probability of different sowing time outcomes in reafforestation programmes, and perhaps for the exploration of the question of emergence time and fitness to a given habitat.

Nevertheless, there are some clear indications for management practices from this work:

1. Field demographic studies indicated that obtaining an abundant flush of germinants was important in securing a reasonable stocking of seedlings at a future date. Even though sometimes of year were identified to be more hazardous emergence times than others, there was a generally compensatory nature among mortality factors.

2. It was also apparent that stochastic events such as severe frosts can be highly influential on regeneration outcomes. Examination of intra- and inter-provenance variability indicates that germination characteristics display a high degree of variability in dormancy and ability to germinate under water stress, variability which disperses emergence in time. Autumn sowings, which correspond to the timing of natural seedfall, result in a spread of emergence times split between that autumn and the subsequent spring. Late winter and spring times of sowing result in emergence concentrated in one flush in the spring. While the latter pattern of emergence may result in greater cumulative emergence of some sites, it may be an inherently more risky management strategy.

3. Modelling indicates that increasing the proportion of 'safe' sites for germination increases the probability that some seeds will survive adverse conditions. Seedbed heterogeneity, and the number of safe sites, decreases with seedbed age. Prompt sowing of sites following seedbed preparation increased cumulative seed germination. The longer the time elapsed since seedbed preparation the greater the relative difference between the number of seedlings observed on the recently prepared, as opposed to old, seedbeds. Similarly, seedbed preparation techniques, such a mechanical disturbance, that maximise seedbed heterogeneity at the local scale (tens of centimetres) may facilitate regeneration on adverse sites.

4. Modelling suggests that different proportions of seed dormancy impart different levels of fitness to different environments. Ensuring that seed sown onto a site is collected from as climatically similar a site as possible, preferably the same site, is important to minimise the probability of regeneration failure (as well as, perhaps primarily, ensuring conservation of genetic resources).

5. Significant variation in germination characteristics reside between provenances, between trees within provenances and within trees. Seed collection must ensure that all levels of genetic variation is maintained. Sowing of seed collected from only a few trees may affect the genetic resources of a site.

6. Very little seed remains in the soil more than one spring and one autumn after the time of sowing. Seedlings that have developed beyond the cotyledonary stage are moderately robust, and, in the absence of particularly severe frosts or droughts, extensive mortality is unlikely. Surveying of artificially regenerated areas within 12-24 months after sowing is probably adequate to assess accurately stocking and reafforestation success since further regeneration is unlikely and mortality effects, with the possible exception of browsing and insect impacts, are likely to be minor.

9.3 Concluding remarks

This study has used a variety of methods - laboratory studies, field-based demographic studies and mathematical modelling - to investigate aspects of the reproductive ecology of *E. delegatensis*. Each of these methodologies has advantages and disadvantages relative to the others. Field-based demographic

studies are correlative rather than experimental in approach and, consequently, inferences drawn from data may be weak, and a number of explanations of plant response may appear equally valid. Direct experimentation in the laboratory avoids many of the problems associated with confounding effects and nuisance covariates and may clearly highlight causal relationships. Unless effects can be demonstrated to operate in the field, however, inferences entirely reliant upon laboratory-based studies may lack credibility. Mathematical models can be used to test logical consequences of assumptions about biological systems and may allow us to go, via simulation, beyond our data to explore hypothetical situations. Without a sound physiological basis, the models of biological and ecological systems are susceptible to criticisms of "adaptive story-telling" or, as put another way by Scheid (1987), "... with five variables you can construct an elephant, with six he [sic] will wave his [sic] trunk". Applied jointly in the current study these methodologies have proven complementary: with laboratory and glasshouse experiments revealing the underlying causal factors of seed germination and seedling mortality, field work confirming the significance of factors in the real world and highlighting the importance of stochastic events, and modelling allowing the exploration of the implications of these for system management, manipulation and the prediction of the change of the system with time.

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Appendix 1: Summary of germination tests

Appendix 1a. Water Potential Experiments

Seedlot	Matric Potential (MPa)	Temp. (°C)	Reps	Germ. Capacity (%)	Standard Error (95% CI)	Germ. Rate (1/t50)	Standard Error (95% CI)
117	-0.500	20	3	31.1	2.99	0.071	0.0051
117	-0.250	20	3	96.7	19.00	0.110	0.0049
117	-0.100	20	3	95.0	9.62	0.110	0.0072
117	-0.010	20	3	82.9	5.98	0.124	0.0113
117	0.000	20	3	96.0	1.11	0.151	0.0101
m32	-0.500	20	4	5.6	3.56	0.030	0.0183
m32	-0.250	20	4	31.6	3.56	0.069	0.0019
m32	-0.100	20	4	29.8	3.04	0.087	0.0088
m32	-0.010	20	4	45.0	8.51	0.081	0.0026
m32	0.000	20	4	40.0	4.65	0.082	0.0100
m36	-0.500	12.5	5	1.4	1.49	0.007	0.0071
m36	-0.500	17.5	5	7.4	2.04	0.072	0.0098
m36	-0.500	20	5	8.3	1.83	0.069	0.0216
m36	-0.500	22.5	5	2.9	1.40	0.067	0.0370
m36	-0.500	25	5	0.0	0.00	0.000	0.0000
m36	-0.250	12.5	5	2.2	1.49	0.026	0.0166
m36	-0.250	17.5	5	27.2	2.53	0.073	0.0025
m36	-0.250	20	5	16.5	2.18	0.100	0.0070
m36	-0.250	22.5	5	10.4	1.83	0.134	0.0144
m36	-0.250	25	5	2.2	1.49	0.021	0.0126
m36	-0.100	12.5	5	15.7	1.40	0.054	0.0070
m36	-0.100	17.5	5	32.8	2.18	0.097	0.0031
m36	-0.100	20	5	23.9	0.91	0.113	0.0096
m36	-0.100	22.5	5	24.6	2.79	0.115	0.0119
m36	-0.100	25	5	3.0	0.75	0.050	0.0132
m36	-0.075	20	5	32.8	2.18	0.110	0.0128
m36	-0.050	20	5	35.1	1.49	0.114	0.0100
m36	-0.025	20	5	35.8	2.24	0.111	0.0080
m36	-0.010	12.5	5	14.2	0.75	0.066	0.0043
m36	-0.010	17.5	5	41.0	4.09	0.111	0.0051
m36	-0.010	20	5	41.0	3.12	0.113	0.0084
m36	-0.010	22.5	5	24.6	2.53	0.109	0.0060
m36	-0.010	25	5	5.2	0.91	0.074	0.0020
m36	-0.0075	20	5	39.6	1.49	0.110	0.0118
m36	-0.005	20	5	43.3	2.24	0.129	0.0032
m36	-0.001	20	5	39.6	0.91	0.118	0.0045
m36	0.000	12.5	5	16.4	2.53	0.053	0.0007
m36	0.000	17.5	5	46.3	3.25	0.106	0.0079
m36	0.000	20	5	50.8	4.51	0.122	0.0097
m36	0.000	22.5	5	20.9	2.53	0.142	0.0138
m36	0.000	25	5	9.0	0.91	0.081	0.0021
m38	-0.500	20	4	5.6	2.02	0.049	0.0169
m38	-0.250	20	4	20.9	1.97	0.078	0.0036
m38	-0.100	20	4	19.7	4.37	0.070	0.0238
m38	-0.010	20	4	24.9	5.19	0.103	0.0098
m38	0.000	20	4	33.8	3.83	0.092	0.0036

Appendix 1a (cont)

Seedlot	Matric Potential (MPa)	Temp. (°C)	Reps	Germ. Capacity (%)	Standard Error (95% CI)	Germ. Rate (1/t50)	Standard Error (95% CI)
m50	-0.500	20	4	1.2	1.18	0.015	0.0146
m50	-0.250	20	4	9.4	1.93	0.055	0.0139
m50	-0.100	20	4	18.9	6.68	0.07	0.0136
m50	-0.010	20	4	39.0	5.24	0.079	0.0119
m50	0.000	20	4	42.5	6.95	0.077	0.0065

Appendix 1b. Temperature and Stratification Experiments

* 20°C 14 hour day & 10°C 10 hour night

** 20°C 18 hour day & 15°C 6 hour night

Seedlot	Duration Strat. (days)	Temp. (°C)	Reps	Germ. Capacity (%)	Standard Error (95% CI)	Germ. Rate (1/t50)	Standard Error (95% CI)
117	0	5	4	95.7	1.81	0.015	0.0001
117	0	12.5	4	93.5	2.81	0.065	0.0019
117	0	17.5	4	90.1	2.66	0.108	0.0053
117	0	20	4	96.0	1.11	0.102	0.0022
117	0	22.5	4	54.0	1.65	0.100	0.0058
117	14	20	4	98.2	2.66	0.202	0.0094
117	28	12.5	4	100.0	2.95	0.151	0.0053
117	28	17.5	4	87.4	1.83	0.235	0.0138
117	28	20	4	91.3	0.41	0.258	0.0043
117	28	22.5	4	99.4	7.47	0.288	0.0094
117	56	20	4	92.1	1.08	0.766	0.0476
m32	0	5	4	65.6	4.5	0.011	0.0002
m32	0	12.5	4	9.9	2.14	0.050	0.0046
m32	0	17.5	4	59.6	3.3	0.073	0.0042
m32	0	20	4	48.2	1.49	0.075	0.0015
m32	0	22.5	4	16.9	2.07	0.077	0.0078
m32	14	20	4	58.1	4.75	0.129	0.0009
m32	28	12.5	4	60.6	7.19	0.089	0.0032
m32	28	17.5	4	100	4.69	0.137	0.0097
m32	28	20	4	84.5	5.03	0.160	0.0075
m32	28	22.5	4	58.6	3.8	0.160	0.0062
m32	56	20	4	86.5	7.14	0.264	0.0179
m36	0	2	4	0.0	0.00	0.000	0.0000
m36	0	5	4	95.3	1.38	0.012	0.0001
m36	0	7.5	4	5.5	0.65	0.019	0.0008
m36	0	12.5	4	21.0	1.41	0.047	0.0006
m36	0	15	8	66.0	0.71	0.064	0.0009
m36	0	20/10*	4	33.8	1.11	0.069	0.0008
m36	0	17.5	4	52.5	2.40	0.078	0.0018
m36	0	20/15**	4	50.0	0.58	0.085	0.0022
m36	0	20	8	50.8	2.02	0.078	0.0026
m36	0	22.5	4	19.5	0.50	0.078	0.0080
m36	0	25	4	9.3	0.63	0.067	0.0028
m36	7	12.5	4	21.5	0.87	0.054	0.0020
m36	7	15	4	76.5	0.65	0.074	0.0019
m36	7	20	4	62.5	0.65	0.105	0.0019
m36	7	25	4	17.0	1.47	0.084	0.0049
m36	14	5	4	95.3	1.38	0.014	0.0001

Appendix 1b. (cont)

Seedlot	Duration Strat. (days)	Temp. (°C)	Reps	Germ. Capacity (%)	Standard Error (95% CI)	Germ. Rate (1/t50)	Standard Error (95% CI)
m36	14	7.5	4	20.5	3.75	0.019	0.0002
m36	14	12.5	4	31.8	0.48	0.063	0.0022
m36	14	15	4	88.5	0.65	0.089	0.0039
m36	14	20/10*	4	48.0	0.85	0.101	0.0012
m36	14	17.5	4	81.0	1.68	0.140	0.0023
m36	14	20/15**	4	80.0	3.81	0.147	0.0046
m36	14	20	5	77.2	1.02	0.175	0.0013
m36	14	22.5	4	51.8	1.32	0.153	0.0068
m36	14	25	4	20.8	0.25	0.089	0.0036
m36	28	5	4	95.3	1.38	0.018	0.0001
m36	28	7.5	4	33.3	0.85	0.023	0.0014
m36	28	12.5	4	46.3	0.85	0.072	0.0028
m36	28	15	4	86.0	0.41	0.097	0.0021
m36	28	20/10*	4	77.0	1.41	0.142	0.0054
m36	28	17.5	4	86.3	1.03	0.183	0.0045
m36	28	20/15**	4	83.8	1.03	0.213	0.0154
m36	28	20	4	90.8	0.48	0.233	0.0077
m36	28	22.5	4	77.3	2.32	0.203	0.0072
m36	28	25	4	58.0	1.22	0.119	0.0138
m36	56	5	4	95.3	1.38	0.035	0.0005
m36	56	7.5	4	66.0	0.91	0.050	0.0024
m36	56	12.5	4	67.5	0.87	0.103	0.0077
m36	56	15	4	87.0	2.80	0.148	0.0100
m36	56	20/10*	4	88.8	1.97	0.227	0.0057
m36	56	17.5	4	95.8	1.93	0.294	0.0165
m36	56	20/15**	4	109.3	2.29	0.373	0.0190
m36	56	20	4	101.0	1.00	0.465	0.0243
m36	56	22.5	4	82.8	1.93	0.351	0.0112
m36	56	25	4	93.0	2.35	0.243	0.0192
m38	0	5	4	82.8	7.34	0.012	0.0001
m38	0	12.5	4	45.8	9.77	0.051	0.0050
m38	0	17.5	4	66.5	4.44	0.081	0.0011
m38	0	20	4	69.5	2.83	0.085	0.0054
m38	0	22.5	4	23.7	1.71	0.077	0.0100
m38	14	20	4	74.0	4.68	0.133	0.0044
m38	28	12.5	4	82.8	8.71	0.107	0.0033
m38	28	17.5	4	100	8.04	0.183	0.0049
m38	28	20	4	84.3	3.72	0.213	0.0061
m38	28	22.5	4	91.7	4.68	0.223	0.0049
m38	56	20	4	91.7	4.68	0.463	0.0151
m50	0	5	4	64.7	2.96	0.011	0.0002
m50	0	12.5	4	18.8	4.51	0.056	0.0039
m50	0	17.5	4	39.4	5.12	0.064	0.0037
m50	0	20	4	38.2	2.23	0.061	0.0037
m50	0	22.5	4	21.8	3.89	0.063	0.0042
m50	14	20	4	42.3	3.04	0.096	0.0039
m50	28	12.5	4	50.0	3.09	0.080	0.0044
m50	28	17.5	4	75.9	6.03	0.103	0.0061
m50	28	20	4	58.3	1.12	0.124	0.0108
m50	28	22.5	4	57.6	4.35	0.162	0.0145
m50	56	20	4	100	3.11	0.194	0.0061

Appendix 1c. Strengthened Dormancy Experiments.
all germination completed at 20°C.
all experiments using M36

Prior Treatments	Duration Strat. (days)	PostStrat. Treatments	Reps	Germ. Capacity (%)	Standard Error (95% CI)	Germ. Rate (1/t50)	Standard Error (95% CI)
-	0	24 hours at 35°C	4	45.0	2.12	0.069	0.0019
-	7	24 hours at 35°C	4	48.8	0.85	0.073	0.0060
-	14	24 hours at 35°C	4	62.0	1.22	0.104	0.0049
-	28	24 hours at 35°C	4	77.3	1.55	0.112	0.0060
-	56	24 hours at 35°C	4	92.3	2.78	0.196	0.0045
8 hours at 35°C	0	-	4	43.0	1.91	0.081	0.0046
48 hours at 35°C	0	-	4	39.8	1.65	0.070	0.0049
8 hours at 35°C	14	-	4	72.0	1.78	0.108	0.0033
8 hours at 35°C	28	-	4	71.0	3.14	0.137	0.0038
24 hours at 35°C	28	-	4	64.8	2.60	0.138	0.0078
48 hours at 35°C	28	-	4	75.7	1.74	0.145	0.0174
8 hours at 35°C	56	-	4	84.3	4.39	0.309	0.0009
24 hours at 25°C	0	-	4	49.8	0.63	0.074	0.0040
24 hours at 30°C	0	-	4	43.5	1.19	0.074	0.0060

Appendix 1d. Wetting and drying Experiments.
all germination at 20°C.
all experiments using M36
w24=wet for 24 hours, d24=dry 24 hours, w=wet thereafter, etc.

Treatment	Reps	Germ. Capacity (%)	Standard Error (95% CI)
w24-d24-w	5	66.0	5.47
w48-d24-w	5	59.0	7.42
w60-d24-w	5	42.0	5.70
w80-d24-w	5	50.0	5.00
w120-d24-w	5	44.0	4.18
w140-d24-w	5	20.0	7.08
w160-d24-w	5	16.0	5.48
w24-d48-w24-d24-w	5	55.0	5.00
w24-d24-w24-d24-w12-d24-w	5	50.0	6.12
w48-d72-w	5	56.0	9.62
w48-d168-w	5	65.0	7.90
w48-d48-w	5	52.0	4.50
continuously wet	6	65.8	8.37

Appendix 1e. Intra- and inter-provenance variation experiments.
all germination in laboratory held between 15-22.5°C.
water potentials applied osmotically using poly-ethylene glycol 6000 MW
Stratification at constant 5°C
Germination media was filter papers within petri-dishes

Site	Tree	Duration Strat. (days)	Water Pot. (MPa)	Reps	Germ. Capacity (%)	Standard Error (95% CI)
Bicheno	1	0	0	4	79.5	1.1
Bicheno	1	14	0	4	69.4	4.1
Bicheno	1	28	0	4	87.9	2.8
Bicheno	1	56	0	4	100.0	3.8
Bicheno	2	0	0	4	55.3	4.2
Bicheno	2	14	0	4	109.7	13.5
Bicheno	2	28	0	4	92.7	8.2
Bicheno	2	56	0	4	100.0	3.6
Bicheno	3	0	0	4	127.7	17.6
Bicheno	3	14	0	4	135.1	7.8
Bicheno	3	28	0	4	124.4	4.7
Bicheno	3	56	0	4	100.0	22.1
Bicheno	4	0	0	4	91.2	5.2
Bicheno	4	14	0	4	90.5	6.6
Bicheno	4	28	0	4	103.6	2.4
Bicheno	4	56	0	4	100.0	11.8
Bicheno	5	0	0	4	74.6	13.3
Bicheno	5	14	0	4	91.5	9.8
Bicheno	5	28	0	4	104.2	12.7
Bicheno	5	56	0	4	100.0	17.4
Bicheno	6	0	0	4	69.0	3.1
Bicheno	6	14	0	4	92.2	9.8
Bicheno	6	28	0	4	87.1	11.4
Bicheno	6	56	0	4	100.0	12.2
Ben Nevis	1	0	0	4	18.5	5.6
Ben Nevis	1	14	0	4	65.3	2.9
Ben Nevis	1	28	0	4	76.1	2.3
Ben Nevis	1	56	0	4	100.0	2.0
Ben Nevis	2	0	0	4	47.2	2.3
Ben Nevis	2	14	0	4	81.5	4.1
Ben Nevis	2	28	0	4	101.2	5.1
Ben Nevis	2	56	0	4	100.0	2.7
Ben Nevis	3	0	0	4	15.0	2.7
Ben Nevis	3	14	0	4	50.0	5.3
Ben Nevis	3	28	0	4	94.1	8.6
Ben Nevis	3	56	0	4	100.0	4.1
Ben Nevis	4	0	0	4	13.6	13.6
Ben Nevis	4	14	0	4	68.2	22.7
Ben Nevis	4	28	0	4	86.3	26.1
Ben Nevis	4	56	0	4	100.0	28.2
Ben Nevis	5	0	0	4	31.1	2.6
Ben Nevis	5	14	0	4	97.3	28.2
Ben Nevis	5	28	0	4	87.9	4.6
Ben Nevis	5	56	0	4	100.0	8.4
Ben Nevis	6	0	0	4	34.4	4.2
Ben Nevis	6	14	0	4	117.8	13.8
Ben Nevis	6	28	0	4	100.0	4.2
Ben Nevis	6	56	0	4	100.0	6.4

Appendix 1e. (cont.)

Site	Tree	Duration Strat. (days)	Water Pot. (MPa)	Reps	Germ. Capacity (%)	Standard Error (95% CI)
Bicheno	1	0	0	4	100.0	1.4
Bicheno	1	0	-0.25	4	78.4	2.0
Bicheno	1	0	-0.5	4	24.2	3.1
Bicheno	1	0	-0.75	4	2.1	0.8
Bicheno	2	0	0	4	100.0	7.6
Bicheno	2	0	-0.25	4	45.6	6.5
Bicheno	2	0	-0.5	4	38.2	6.1
Bicheno	2	0	-0.75	4	2.9	1.7
Bicheno	3	0	0	4	100.0	13.8
Bicheno	3	0	-0.25	4	65.0	5.5
Bicheno	3	0	-0.5	4	12.5	7.3
Bicheno	3	0	-0.75	4	2.5	1.6
Bicheno	4	0	0	4	100.0	5.7
Bicheno	4	0	-0.25	4	80.4	6.1
Bicheno	4	0	-0.5	4	65.6	5.1
Bicheno	4	0	-0.75	4	11.6	3.1
Bicheno	5	0	0	4	100.0	17.8
Bicheno	5	0	-0.25	4	71.7	8.9
Bicheno	5	0	-0.5	4	7.5	3.0
Bicheno	5	0	-0.75	4	7.5	3.1
Bicheno	6	0	0	4	100.0	4.5
Bicheno	6	0	-0.25	4	42.5	6.6
Bicheno	6	0	-0.5	4	25.0	4.1
Bicheno	6	0	-0.75	4	1.2	1.2
Ben Nevis	1	0	0	4	100.0	30.4
Ben Nevis	1	0	-0.25	4	10.5	4.8
Ben Nevis	1	0	-0.5	4	6.6	3.9
Ben Nevis	1	0	-0.75	4	0.0	0.0
Ben Nevis	2	0	0	4	100.0	4.8
Ben Nevis	2	0	-0.25	4	16.4	2.3
Ben Nevis	2	0	-0.5	4	4.5	2.7
Ben Nevis	2	0	-0.75	4	0.0	0.0
Ben Nevis	3	0	0	4	100.0	17.8
Ben Nevis	3	0	-0.25	4	7.8	5.0
Ben Nevis	3	0	-0.5	4	0.0	0.0
Ben Nevis	3	0	-0.75	4	0.0	0.0
Ben Nevis	4	0	0	4	100.0	100.0
Ben Nevis	4	0	-0.25	4	100.0	63.8
Ben Nevis	4	0	-0.5	4	0.0	0.0
Ben Nevis	4	0	-0.75	4	0.0	0.0
Ben Nevis	5	0	0	4	100.0	8.3
Ben Nevis	5	0	-0.25	4	21.7	13.0
Ben Nevis	5	0	-0.5	4	4.3	4.3
Ben Nevis	5	0	-0.75	4	0.0	0.0
Ben Nevis	6	0	0	4	100.0	12.2
Ben Nevis	6	0	-0.25	4	16.1	9.7
Ben Nevis	6	0	-0.5	4	25.8	7.4
Ben Nevis	6	0	-0.75	4	3.2	3.2

APPENDIX 2: Floristic survey of experimental sites

List of species found within experimental plots (EP) used for the field demographic study and from nearby randomly located undisturbed sites typical of these experimental plots. Taxonomy follows Buchanan *et al.* 1989.

(Survey and species identification by Ms. Jayne Balmer, Botanist WHA, Department Parks, Wildlife and Heritage).

Life form	SPECIES:	BI25		MC31		
		EP	1	EP	1	2
	Pteridophyta					
F	*Pteridium esculentum*	2	2	+	1	1
	Monocotyledonae					
g	*Agrostis* sp.	-	-	4	-	-
gr	*Dianella revoluta*	1	-	-	-	-
gr	*Diplarrena moraea*	+	+	-	-	-
gr	*Gahnia radula*	-	-	+	-	+
gr	*Holcus lanatus*	-	-	+	-	-
gr	*Lepidosperma laterale*	+	-	-	-	-
gr	Liliaceae sp.	+	-	-	-	-
gr	*Lomandra longifolia*	+	+	+	2	3
g	*Poa* sp	2	+	1	4	3
	Dicotyledonae					
T	*Acacia dealbata*	n	2	n	2	1
T	*Acacia melanoxylon*	-	-	-	-	1
Sm	*Acacia myrtifolia*	+	-	-	-	-
H	*Acaena novae-zelandiae*	-	-	+	+	-
T	*Allocasuarina* sp.	+	-	-	-	-
S	*Amperea xiphoclada*	+	-	-	-	-
T	*Banksia marginata*	n	-	n	3	-
S	*Bedfordia salicina*	-	1	-	-	-
Sm	*Bossiaea cordigera*	-	-	-	-	+
S	*Bossiaea riparia*	-	-	-	+	-
H	*Brachycome* sp.	+	+	-	+	-
H	*Centaurium erythraea*	+	-	n	-	-
H	*Cirsium vulgare*	-	-	1	-	+
S	*Coprosma quadrifida*	-	-	-	-	+
S	*Correa lawrenciana*	-	+	-	-	-
S	*Epacris impressa*	+	-	-	+	-
H	*Epilobium* sp.	+	-	n	-	-

Lifeform	Species	BI25		MC31		
		EP	1	EP	1	2
	Dicotyledonae continued:					
T	*Eucalyptus amygdalina*	-	1	-	+	-
T	*Eucalyptus dalrympleana*	-	-	-	+	-
T	*Eucalyptus delegatensis*	-	-	-	+	3
T	*Eucalyptus obliqua*	n	2	-	-	-
T	*Eucalyptus pauciflora*	-	-	-	3	+
T	*Eucalyptus rubida*	-	-	-	-	+
H	*Geranium solanderi*	-	-	+	+	+
H	*Gnaphalium collinum*	-	-	-	+	-
H	*Gonocarpus serpyllifolius*	+	2	n	-	+
H	*Gonocarpus teucrioides*	1	-	-	-	-
H	*Goodenia lanata*	1	+	-	-	+
H	*Helichrysum scorpioides*	+	-	-	-	+
Sm	*Hibbertia* sp.	1	+	-	+	-
H	*Hydrocotyle* sp.	-	-	-	+	+
H	*Hypericum gramineum*	-	-	+	-	-
H	*Hypochaeris radicata*	+	-	1	-	+
H	*Lagenophora stipitata*	-	-	n	-	+
T	*Leptospermum scoporium*	1	-	-	-	+
S	*Lomatia tinctoria*	+	+	1	+	-
S	*Pimelea linifolia*	-	-	-	+	-
H	*Plantago* sp.	-	-	1	-	-
Sm	*Platylobium triangulare*	+	-	-	-	-
S	*Pultenaea* sp.	1	-	-	-	-
S	*Senecio jacobea*	-	-	1	-	-
H	*Senecio linearifolius*	-	-	-	+	-
H	*Stylidium graminifolium*	+	-	-	+	+
H	*Torazacum officinale*	-	-	2	-	-
H	*Tetratheca pilosa*	+	-	-	-	-
H	*Viola sieberana*	-	-	-	-	+
H	*Viola hederacea*	+	+	n	+	-
H	*Wahlenbergia* sp.	+	-	-	+	+

Key to life forms:

F=fern, g=grass, gr=graminoid, T=tree, S=shrub, Sm=prostrate shrub, H=herb

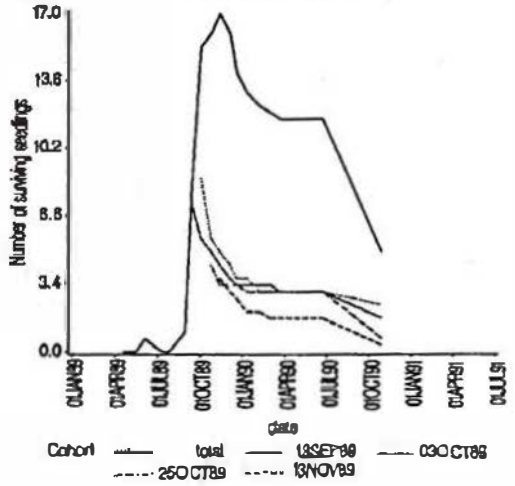
Key to species cover classes:

-=absent, +=<1%, 1=1-4%, 2=5-24%, 3=25-49%, 4=50-74%, n=not in plot but located nearby.

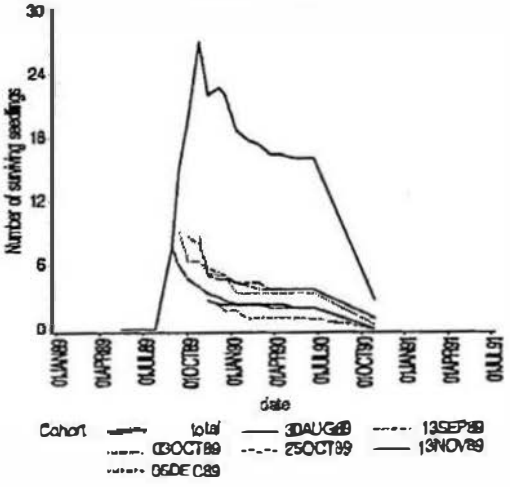
APPENDIX 3. Survivorship curves of field 'cohorts'.

Each 'cohort' consists of all individuals detected at the one scoring time. Each curve represents the mean response of three plots. To avoid graphical confusion only cohorts with more than 5 individuals are displayed. The 'total cohort' is the net population number at the time of scoring.

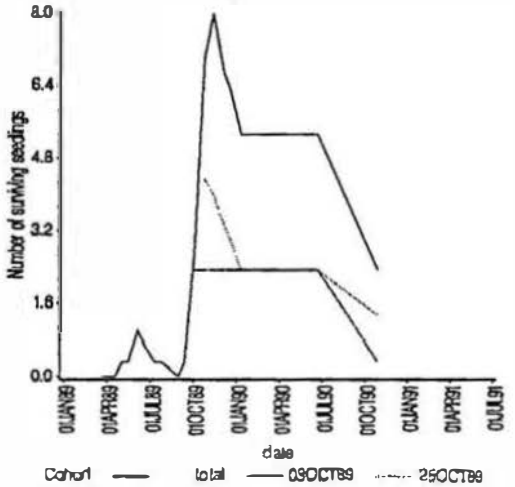
E. amygdalina
MC31
sowing time = 19/4/89



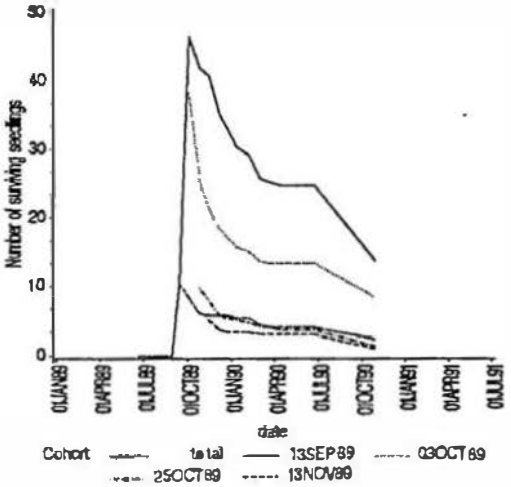
E. amygdalina
MC31
sowing time = 17/5/89



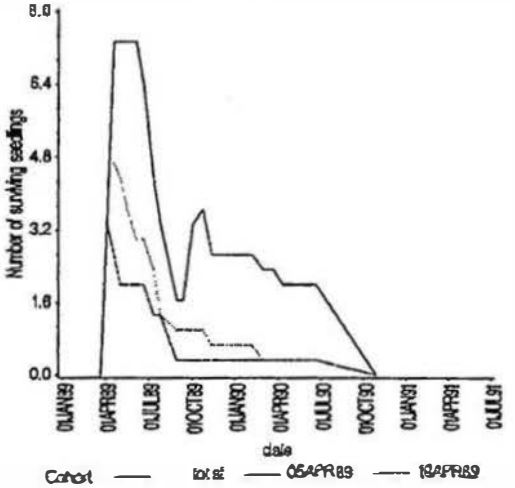
E. amygdalina
MC31
sowing time = 22/3/89



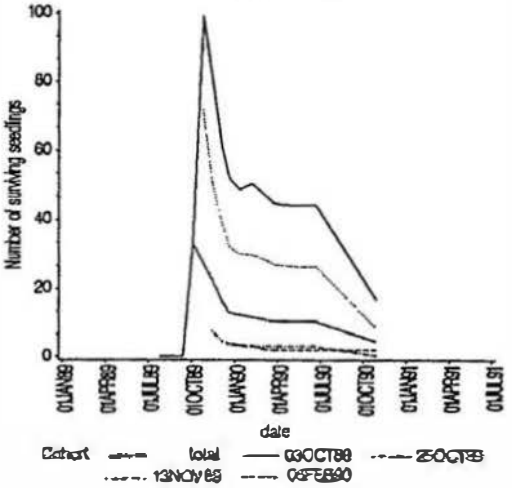
E. amygdalina
MC31
sowing time = 21/6/89

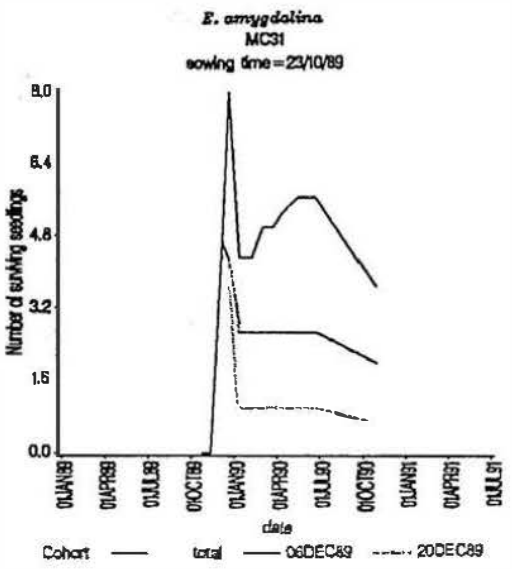


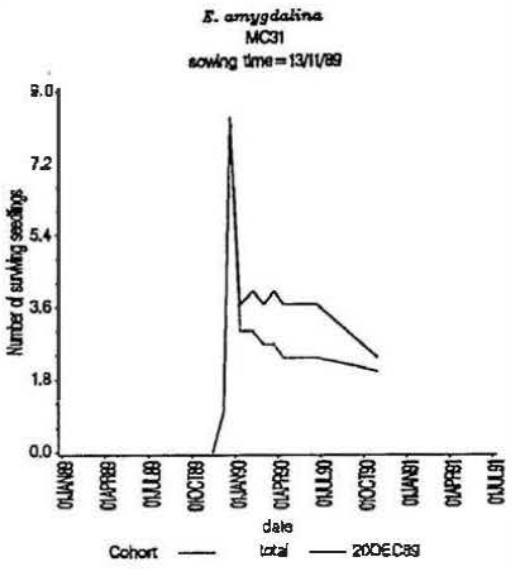
E. amygdalina
MC31
sowing time = 2/3/89

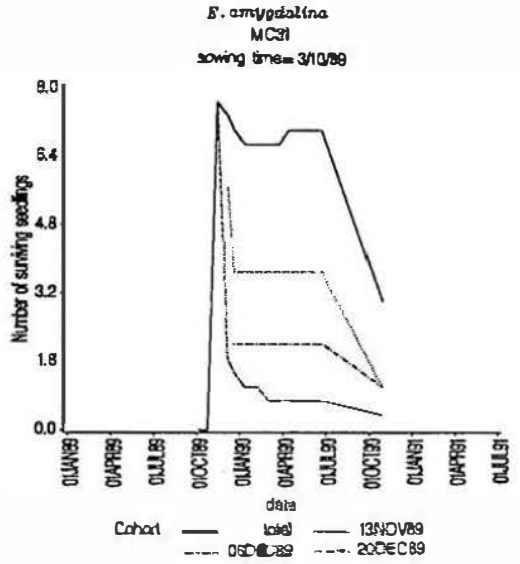


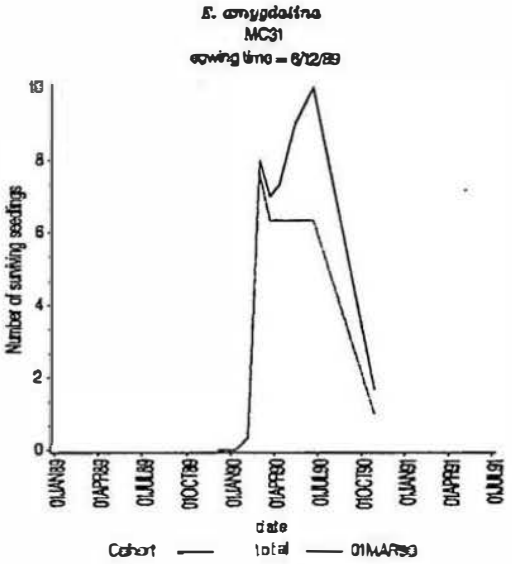
E. amygdalina
MC31
sowing time = 28/7/89

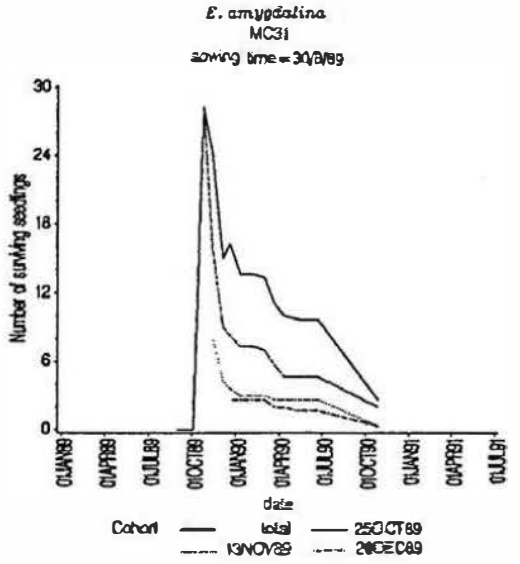


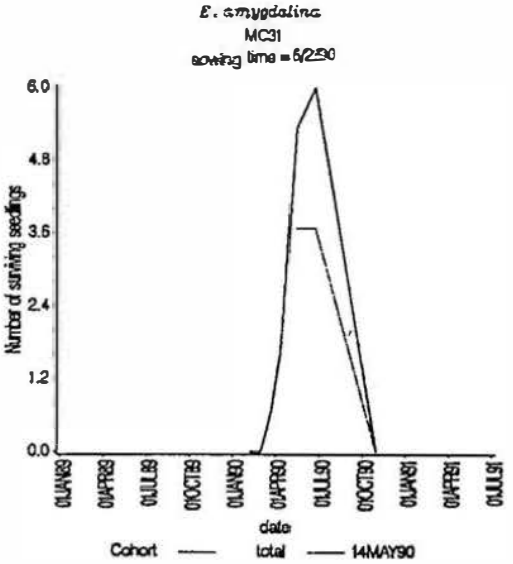

E. amygdalina
MC31
sowing time = 23/10/89
Number of surviving seedlings
8.0
6.4
4.8
3.2
1.6
0.0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
06DEC89
20DEC89

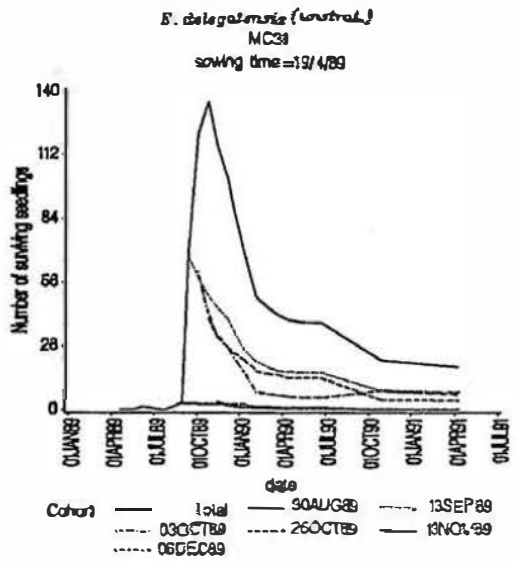

E. amygdalina
MC31
sowing time = 13/11/89
Number of surviving seedlings
9.0
7.2
5.4
3.6
1.8
0.0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
20DEC89

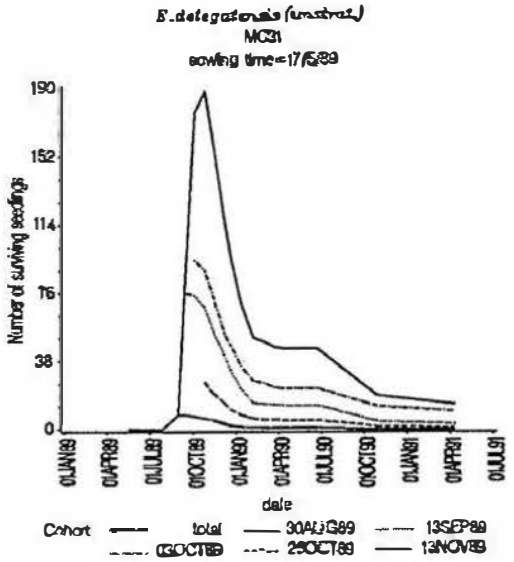

E. amygdalina
MC31
sowing time = 3/10/89
Number of surviving seedlings
8.0
6.4
4.8
3.2
1.8
0.0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
13NOV89
06DEC89
20DEC89

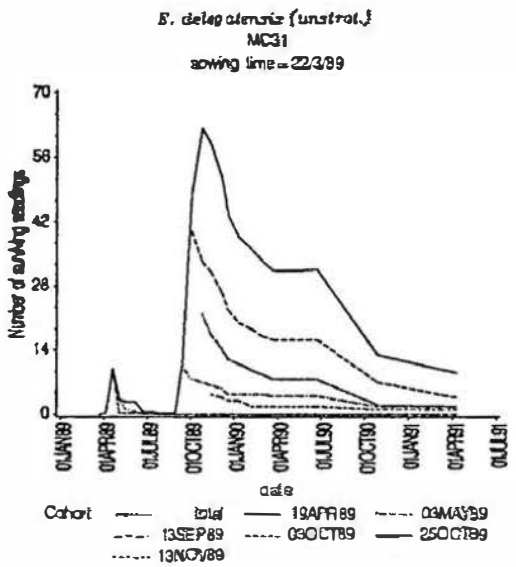

E. amygdalina
MC31
sowing time = 6/12/89
Number of surviving seedlings
10
8
6
4
2
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
01MAR90

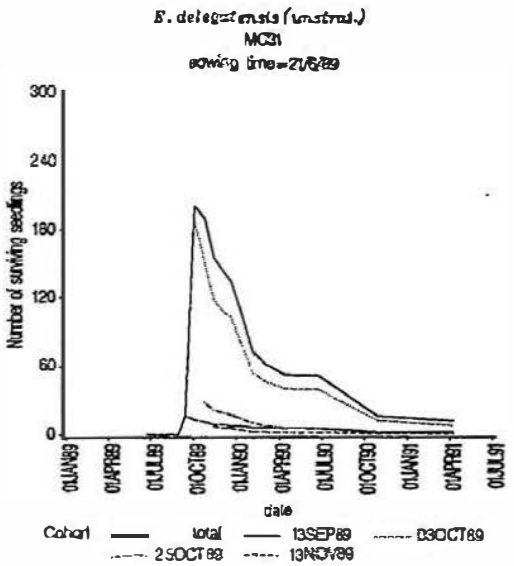

E. amygdalina
MC31
sowing time = 30/9/89
Number of surviving seedlings
30
24
18
12
6
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
25OCT89
13NOV89
20DEC89

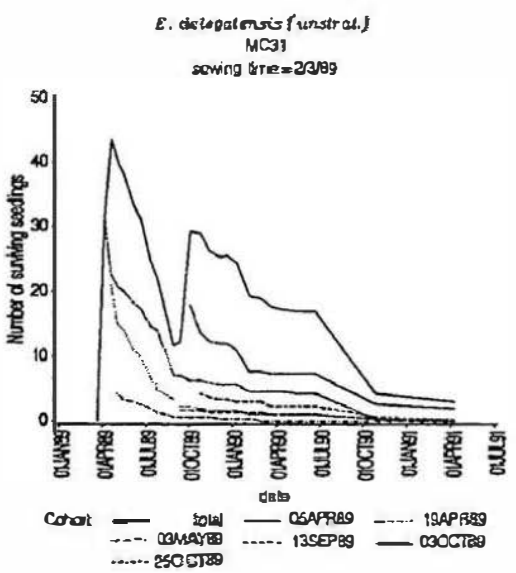

E. amygdalina
MC31
sowing time = 5/2/90
Number of surviving seedlings
6.0
4.8
3.6
2.4
1.2
0.0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
14MAY90

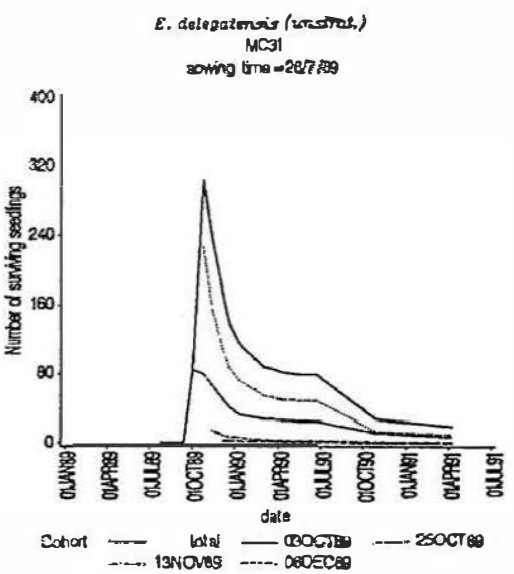

E. delegatensis (unstrat.)
MC31
sowing time=12/4/89
Number of surviving seedlings
140
112
84
56
28
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
30AUG89
13SEP89
03OCT89
26OCT89
13NOV89
06DEC89

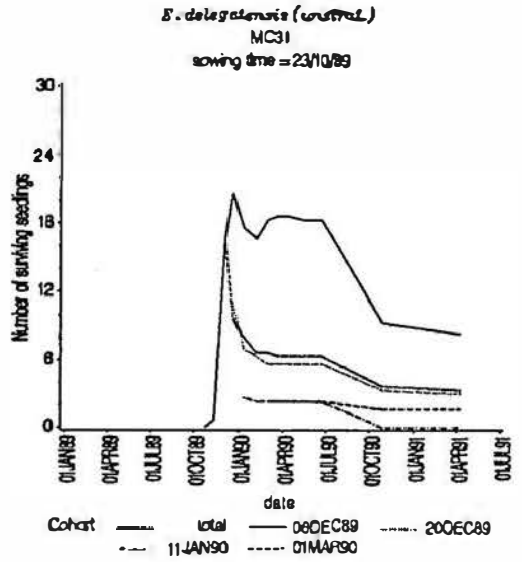

E. delegatensis (unstrat.)
MC31
sowing time=17/5/89
Number of surviving seedlings
190
152
114
76
38
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89

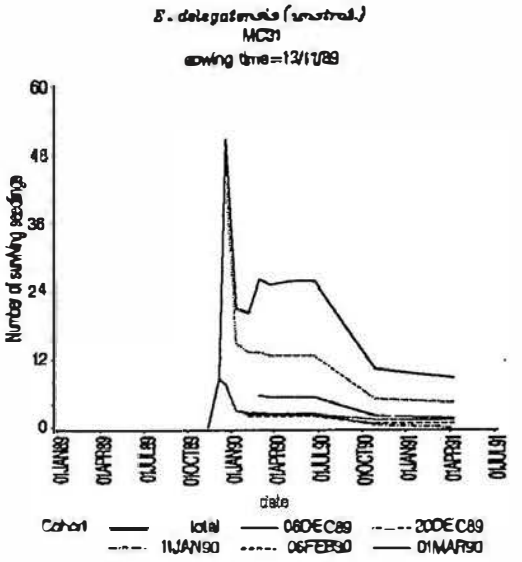

E. delegatensis (unstrat.)
MC31
sowing time=22/3/89
Number of surviving seedlings
70
56
42
28
14
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
19APR89
03MAY89
13SEP89
03OCT89
25OCT89
13NOV89

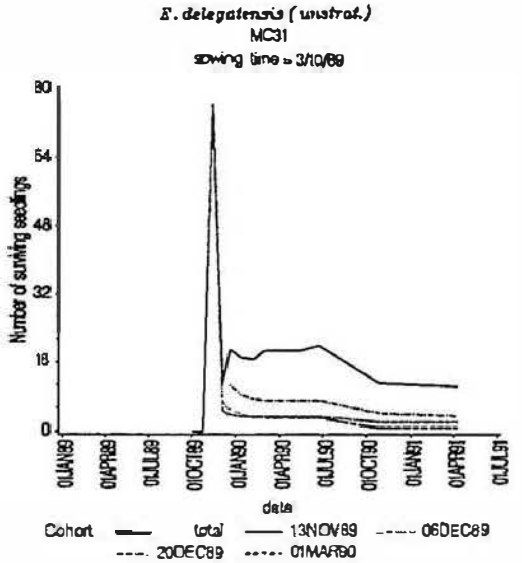

E. delegatensis (unstrat.)
MC31
sowing time=21/6/89
Number of surviving seedlings
300
240
180
120
60
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
13SEP89
03OCT89
25OCT89
13NOV89

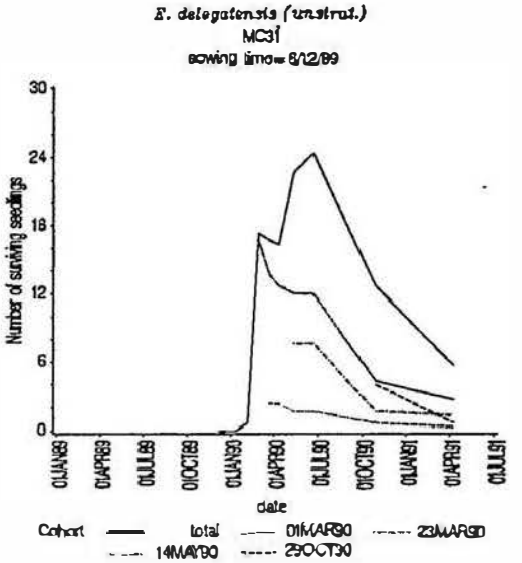

E. delegatensis (unstrat.)
MC31
sowing time=2/3/89
Number of surviving seedlings
50
40
30
20
10
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
05APR89
19APR89
03MAY89
13SEP89
03OCT89
25OCT89

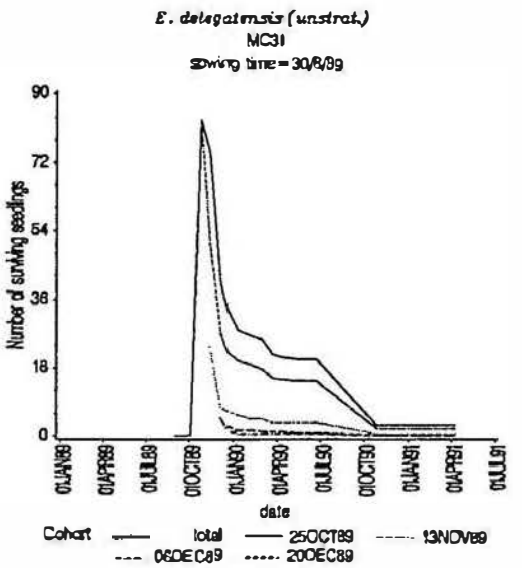

E. delegatensis (unstrat.)
MC31
sowing time=26/7/89
Number of surviving seedlings
400
320
240
160
80
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
03OCT89
25OCT89
13NOV89
06DEC89

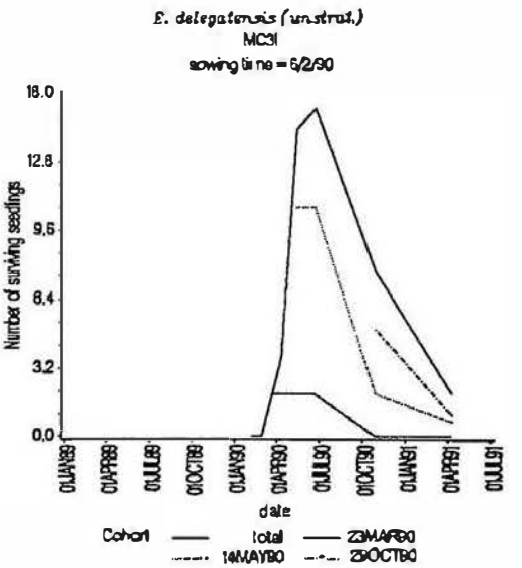

E. delegatensis (unstrat.)
MC31
sowing time = 23/10/89
Number of surviving seedlings
30
24
18
12
6
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
06DEC89
20DEC89
11JAN90
01MAR90

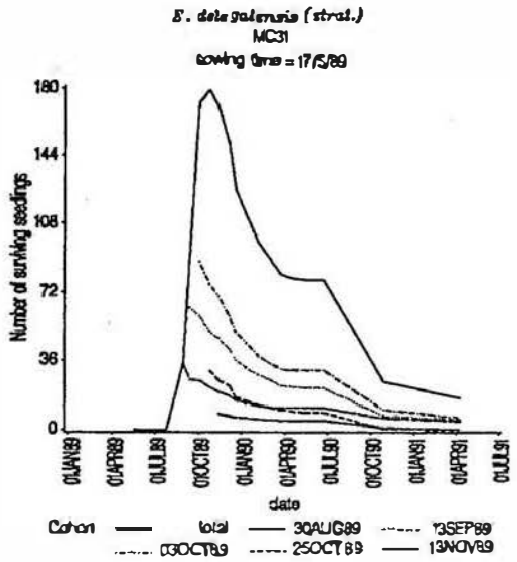

E. delegatensis (unstrat.)
MC31
sowing time = 13/11/89
Number of surviving seedlings
60
48
36
24
12
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
06DEC89
20DEC89
11JAN90
06FEB90
01MAR90

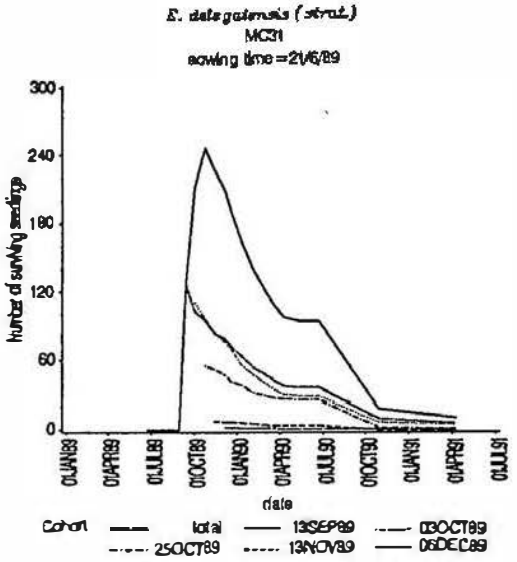

E. delegatensis (unstrat.)
MC31
sowing time = 3/10/89
Number of surviving seedlings
80
64
48
32
16
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
13NOV89
06DEC89
20DEC89
01MAR90

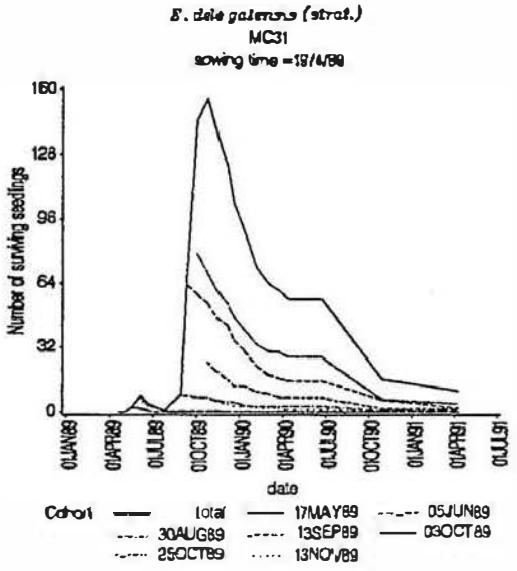

E. delegatensis (unstrat.)
MC31
sowing time = 8/12/89
Number of surviving seedlings
30
24
18
12
6
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
01MAR90
23MAR90
14MAY90
29OCT90

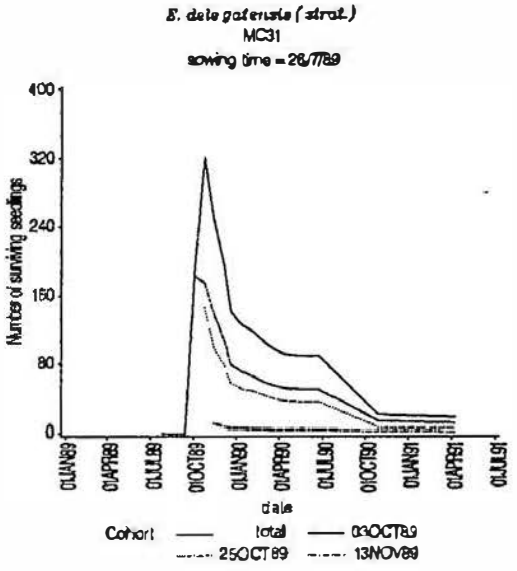

E. delegatensis (unstrat.)
MC31
sowing time = 30/8/89
Number of surviving seedlings
90
72
54
36
18
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
25OCT89
13NOV89
06DEC89
20DEC89

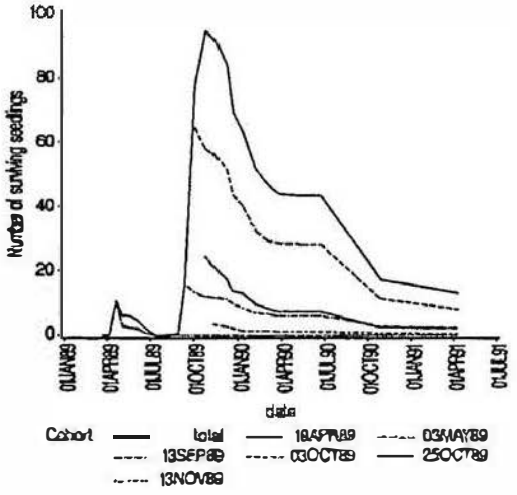

E. delegatensis (unstrat.)
MC31
sowing time = 6/2/90
Number of surviving seedlings
18.0
12.8
9.6
6.4
3.2
0.0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
23MAR90
14MAY90
29OCT90

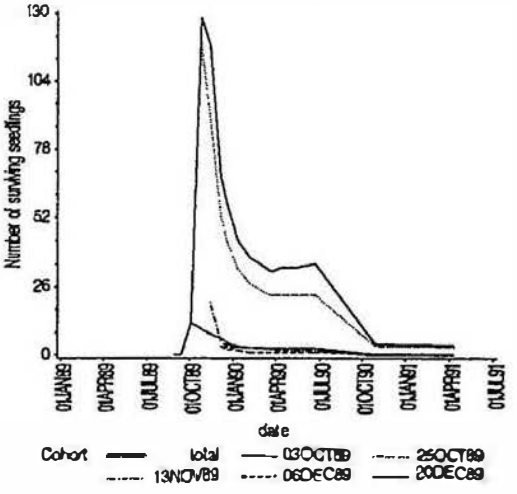

E. delegatensis (strat.)
MC31
sowing time = 17/5/89
Number of surviving seedlings
180
144
108
72
36
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89

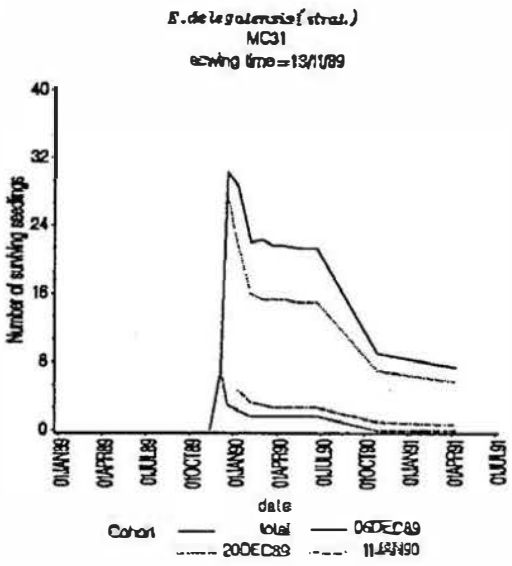

E. delegatensis (strat.)
MC31
sowing time = 21/6/89
Number of surviving seedlings
300
240
180
120
60
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

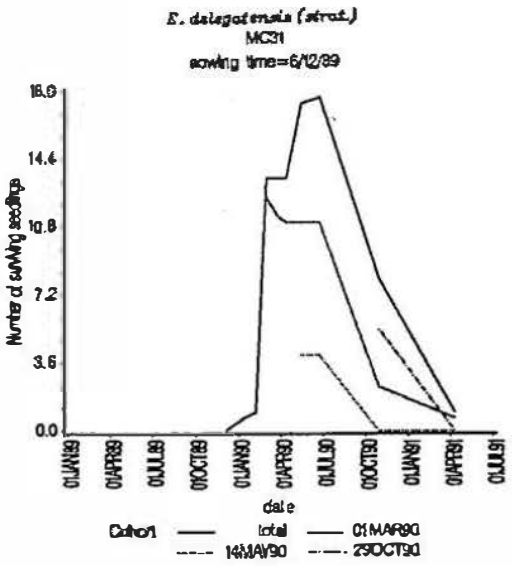

E. delegatensis (strat.)
MC31
sowing time = 19/4/89
Number of surviving seedlings
160
128
96
64
32
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
17MAY89
05JUN89
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89

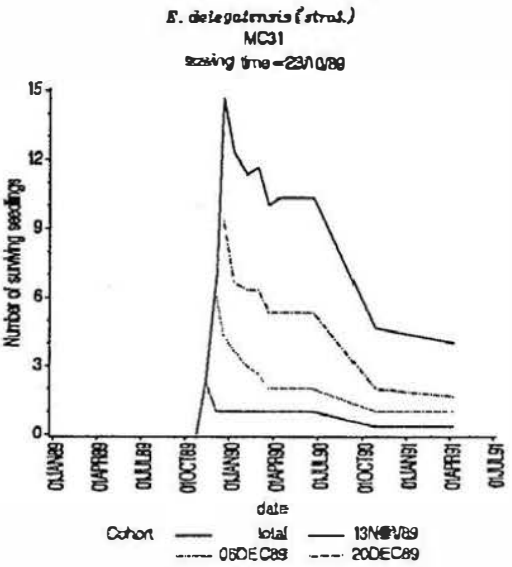

E. delegatensis (strat.)
MC31
sowing time = 26/7/89
Number of surviving seedlings
400
320
240
160
80
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
03OCT89
25OCT89
13NOV89

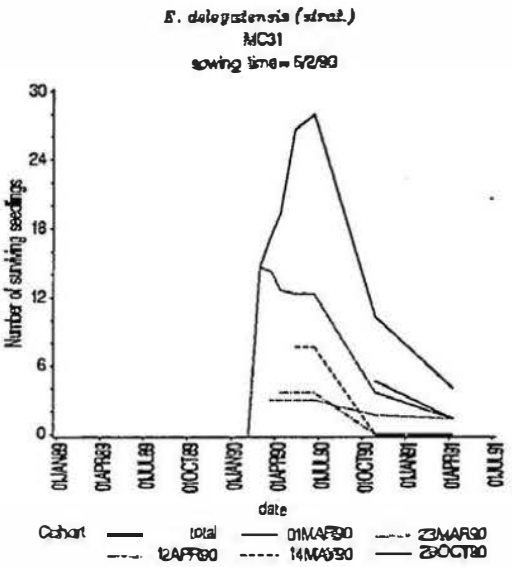

E. delegatensis (strat.)
MC31
sowing time = 22/3/89
Number of surviving seedlings
100
80
60
40
20
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
18APR89
03MAY89
13SEP89
03OCT89
25OCT89
13NOV89

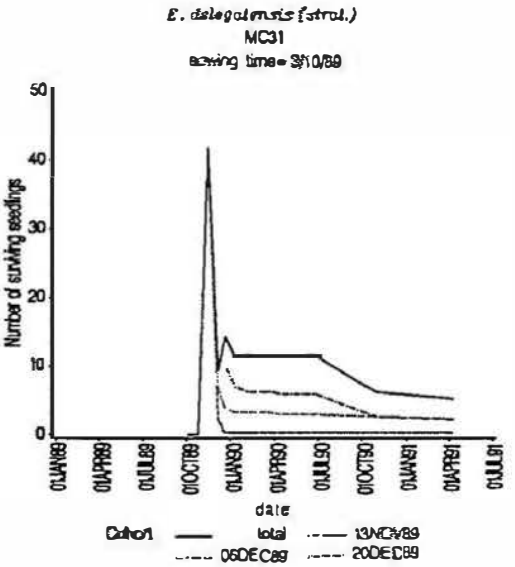

E. delegatensis (strat.)
MC31
sowing time = 30/8/89
Number of surviving seedlings
130
104
78
52
26
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
03OCT89
25OCT89
13NOV89
06DEC89
20DEC89

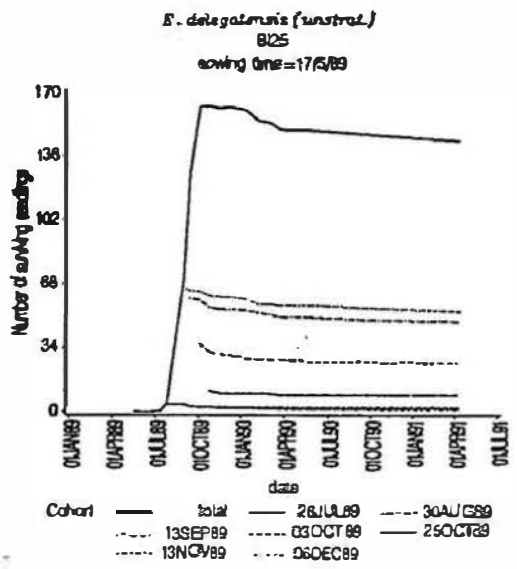

E. delegatensis (strat.)
MC31
sowing time=13/11/89
Number of surviving seedlings
date
Cohort
total
06DEC89
20DEC89
11JAN90

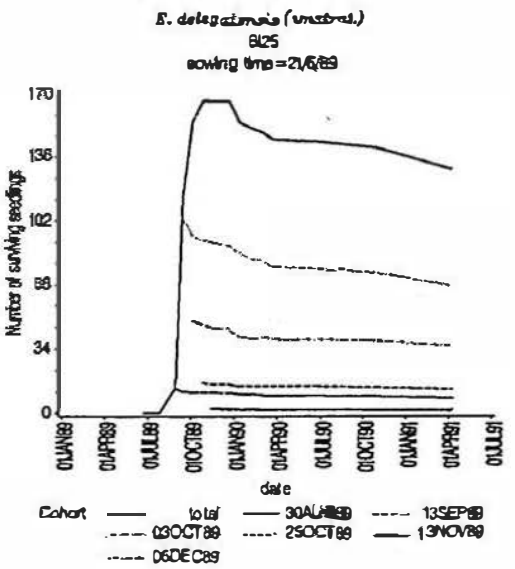

E. delegatensis (strat.)
MC31
sowing time=6/12/89
Number of surviving seedlings
date
Cohort
total
01MAR90
14MAY90
29OCT90

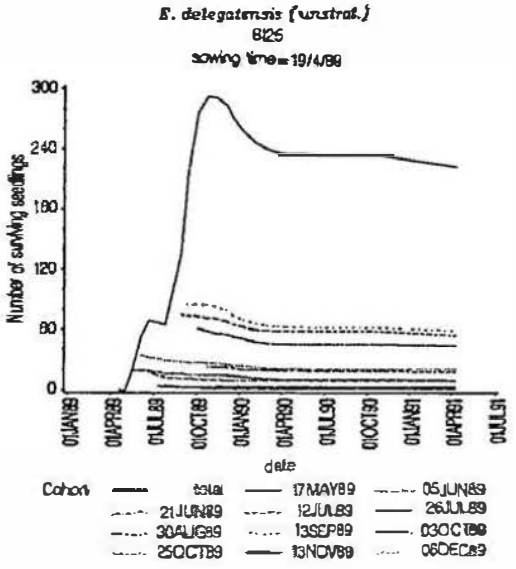

E. delegatensis (strat.)
MC31
sowing time=23/10/89
Number of surviving seedlings
date
Cohort
total
13NOV89
06DEC89
20DEC89

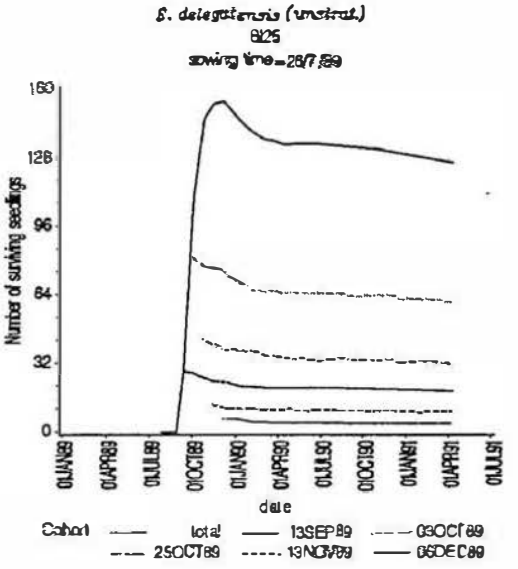

E. delegatensis (strat.)
MC31
sowing time= 5/2/90
Number of surviving seedlings
date
Cohort
total
01MAR90
23MAR90
12APR90
14MAY90
29OCT90

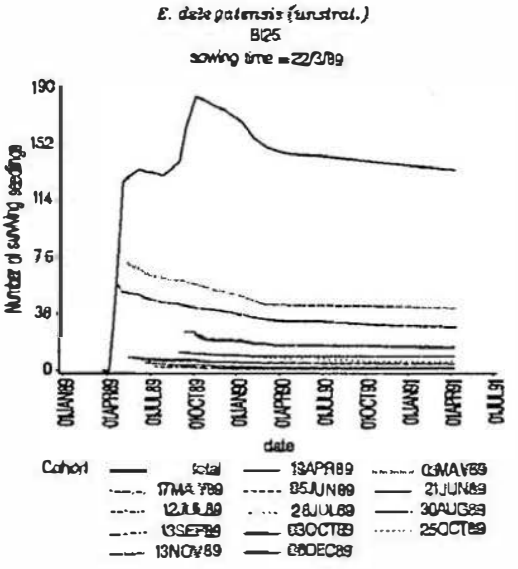

E. delegatensis (strat.)
MC31
sowing time= 3/10/89
Number of surviving seedlings
date
Cohort
total
13NOV89
06DEC89
20DEC89

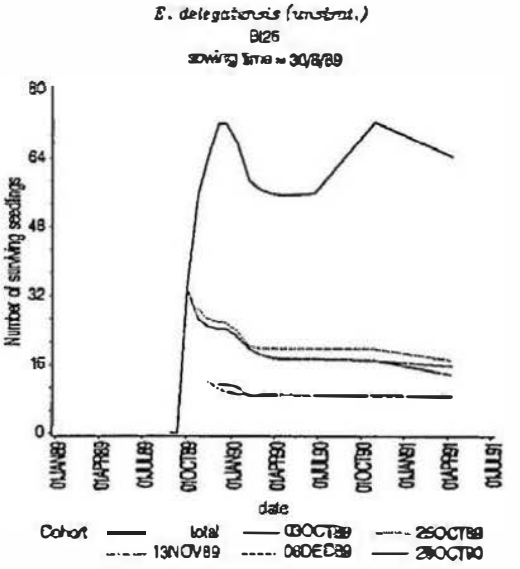

E. delegatensis (unstrat.)
B125
sowing time=17/5/89
Number of surviving seedlings
170
136
102
68
34
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
date
Cohort
total
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

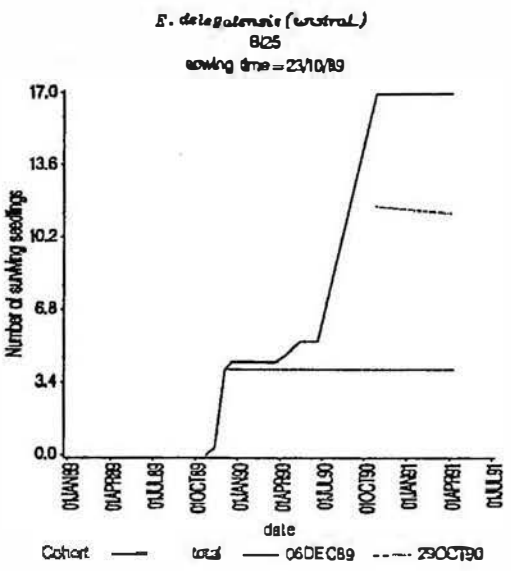

E. delegatensis (unstrat.)
B125
sowing time=21/6/89
Number of surviving seedlings
170
136
102
68
34
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
date
Cohort
total
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

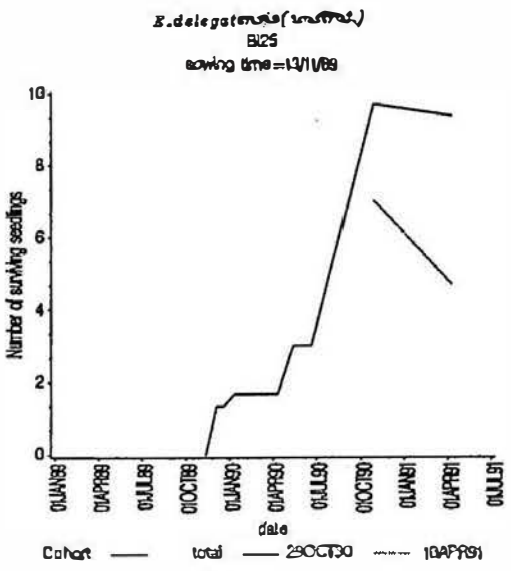

E. delegatensis (unstrat.)
B125
sowing time=19/4/89
Number of surviving seedlings
300
240
180
120
60
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
date
Cohort
total
17MAY89
05JUN89
21JUN89
12JUL89
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

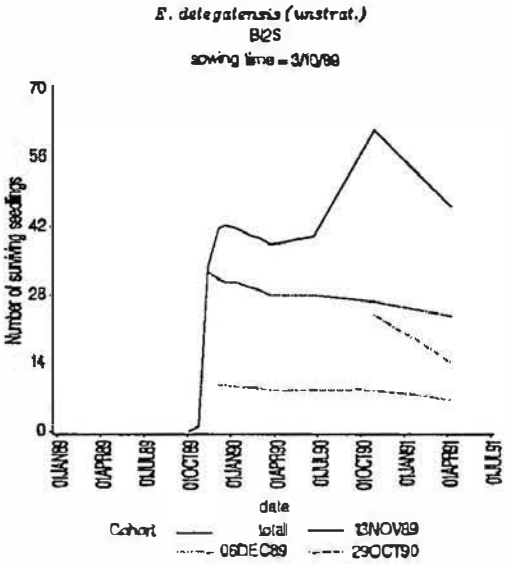

E. delegatensis (unstrat.)
B125
sowing time=26/7/89
Number of surviving seedlings
160
128
96
64
32
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
date
Cohort
total
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

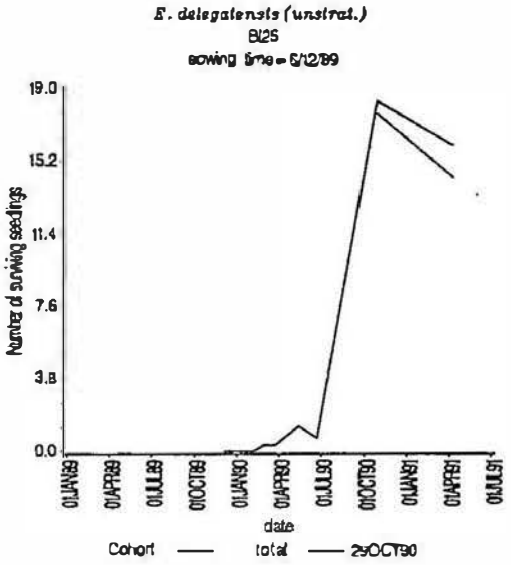

E. delegatensis (unstrat.)
B125
sowing time=22/3/89
Number of surviving seedlings
190
152
114
76
38
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
date
Cohort
total
13APR89
03MAY89
17MAY89
05JUN89
21JUN89
12JUL89
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

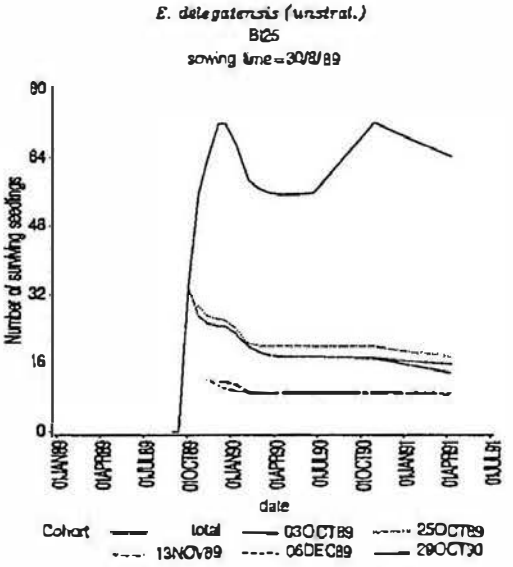

E. delegatensis (unstrat.)
B126
sowing time=30/8/89
Number of surviving seedlings
80
64
48
32
16
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
date
Cohort
total
03OCT89
25OCT89
13NOV89
06DEC89
25OCT90

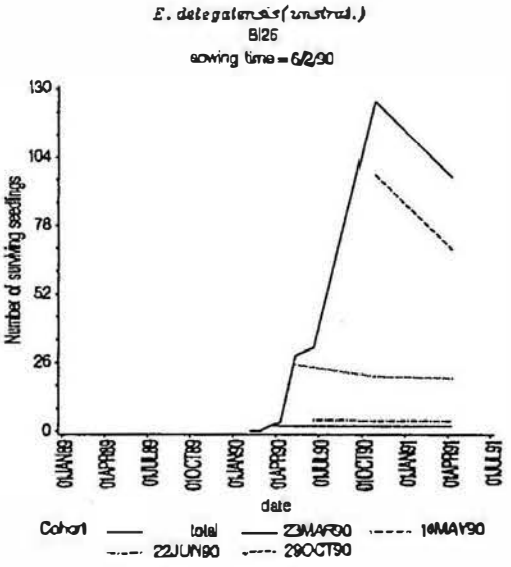

E. delegatensis (unstrat.)
B125
sowing time = 23/10/89
Number of surviving seedlings
17.0
13.6
10.2
6.8
3.4
0.0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
06DEC89
29OCT90

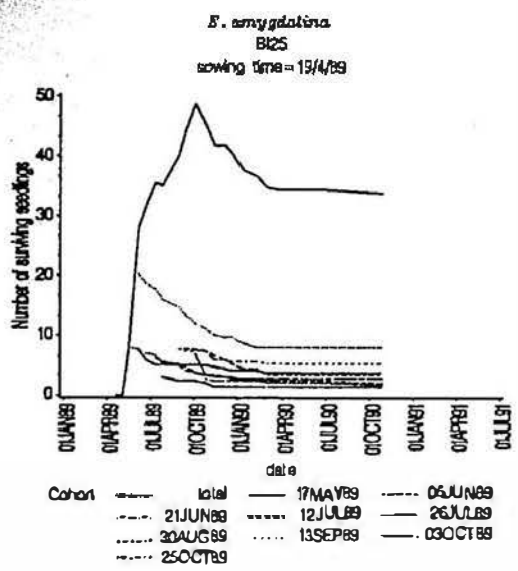

E.delegatensis (unstrat.)
B125
sowing time = 13/11/89
Number of surviving seedlings
10
8
6
4
2
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
29OCT90
10APR91

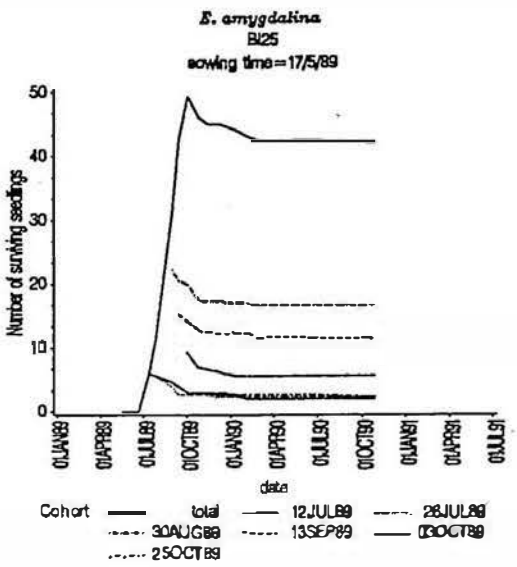

E. delegatensis (unstrat.)
B125
sowing time = 3/10/89
Number of surviving seedlings
70
56
42
28
14
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
13NOV89
06DEC89
29OCT90

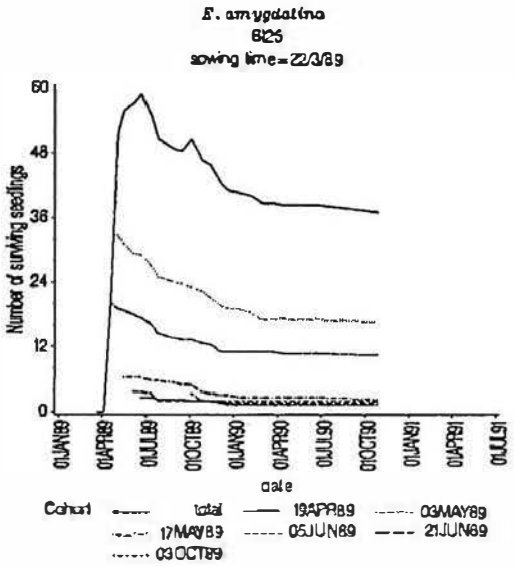

E. delegatensis (unstrat.)
B125
sowing time = 6/12/89
Number of surviving seedlings
19.0
15.2
11.4
7.6
3.8
0.0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
29OCT90

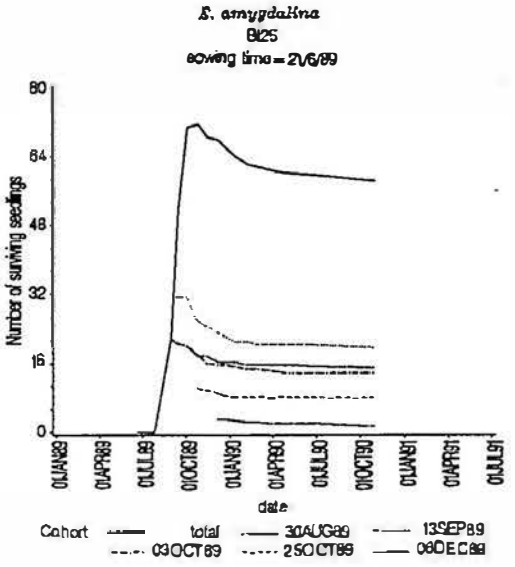

E. delegatensis (unstrat.)
B125
sowing time = 30/8/89
Number of surviving seedlings
80
64
48
32
16
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
03OCT89
25OCT89
13NOV89
06DEC89
29OCT90

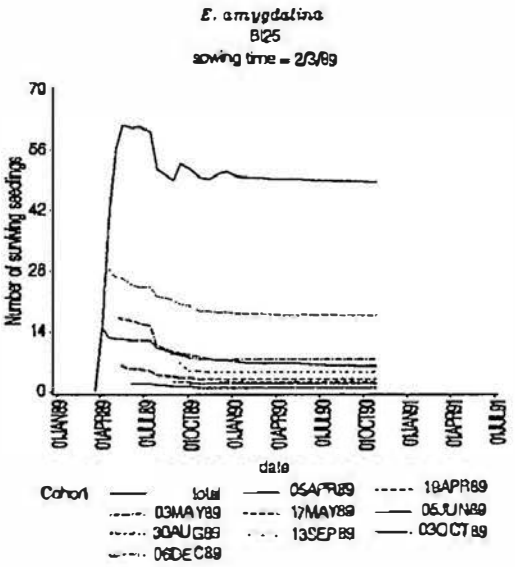

E. delegatensis (unstrat.)
B125
sowing time = 6/2/90
Number of surviving seedlings
130
104
78
52
26
0
01JAN88
01APR88
01JUL88
01OCT88
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
23MAR90
16MAY90
22JUN90
29OCT90

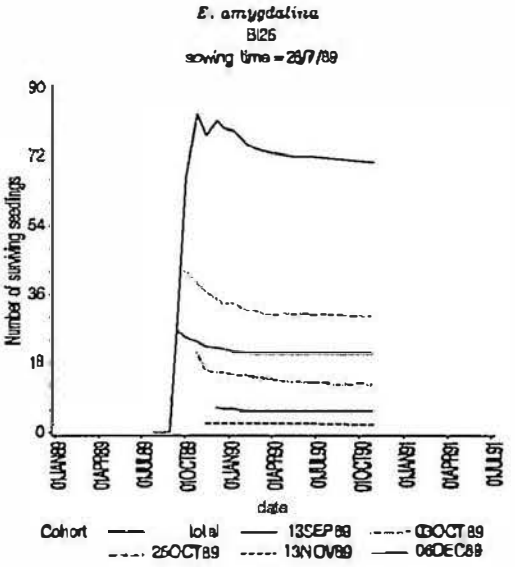

E. amygdalina
B125
sowing time = 19/4/89
Number of surviving seedlings
0
10
20
30
40
50
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
17MAY89
05JUN89
21JUN89
12JUL89
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89

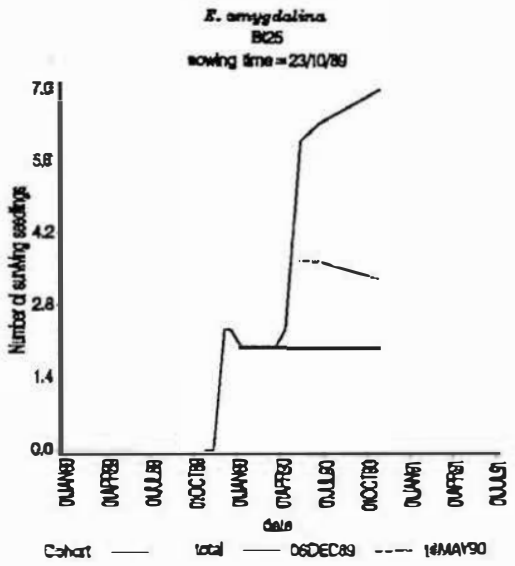

E. amygdalina
B125
sowing time = 17/5/89
Number of surviving seedlings
0
10
20
30
40
50
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
12JUL89
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89

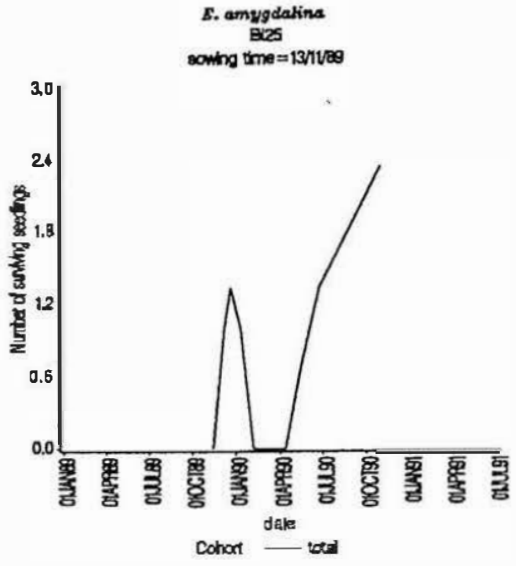

E. amygdalina
B125
sowing time = 22/3/89
Number of surviving seedlings
0
12
24
36
48
60
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
19APR89
03MAY89
17MAY89
05JUN89
21JUN89
03OCT89

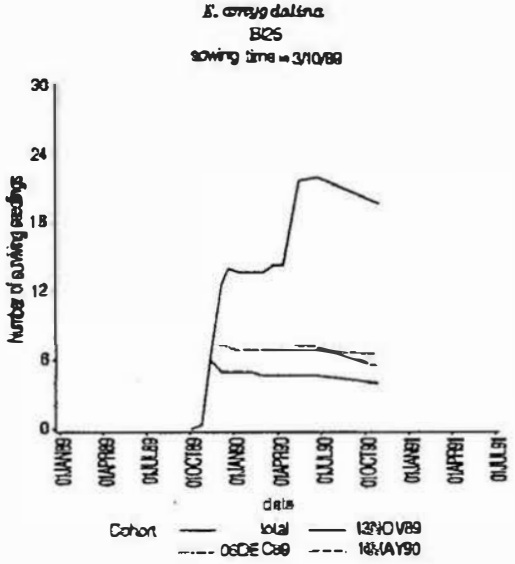

E. amygdalina
B125
sowing time = 21/6/89
Number of surviving seedlings
0
16
32
48
64
80
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
30AUG89
13SEP89
03OCT89
25OCT89
06DEC89

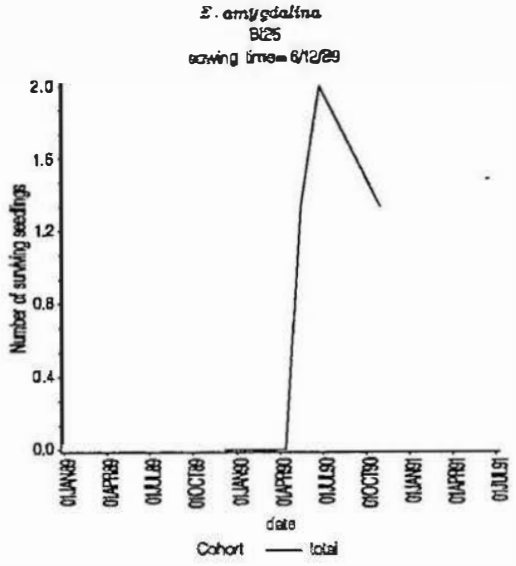

E. amygdalina
B125
sowing time = 2/3/89
Number of surviving seedlings
0
14
28
42
56
70
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
05APR89
18APR89
03MAY89
17MAY89
05JUN89
30AUG89
13SEP89
03OCT89
06DEC89

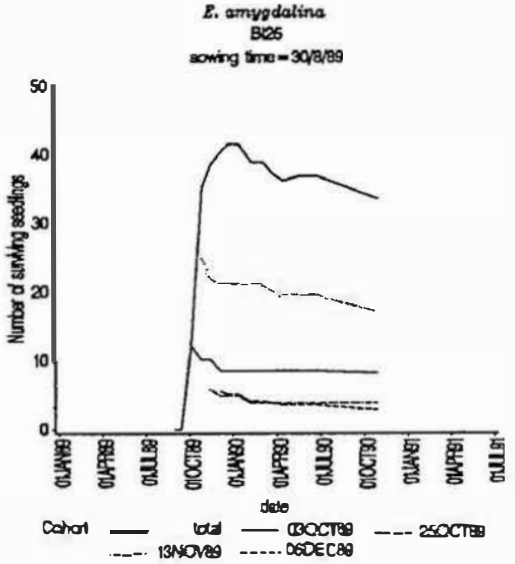

E. amygdalina
B125
sowing time = 28/7/89
Number of surviving seedlings
0
18
36
54
72
90
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

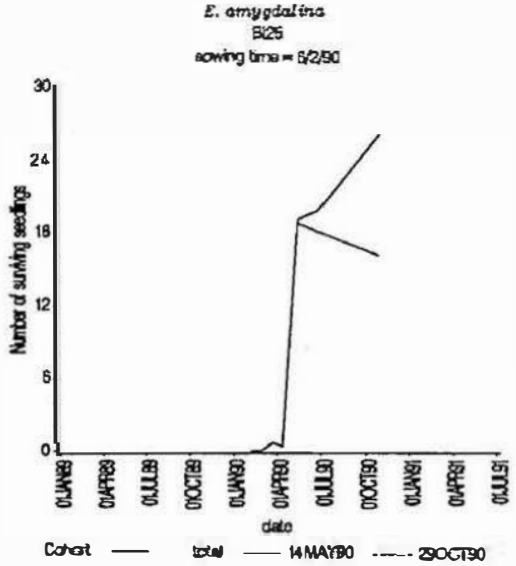

E. amygdalina
Bi25
sowing time = 23/10/89
Number of surviving seedlings
7.0
5.6
4.2
2.8
1.4
0.0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
06DEC89
14MAY90

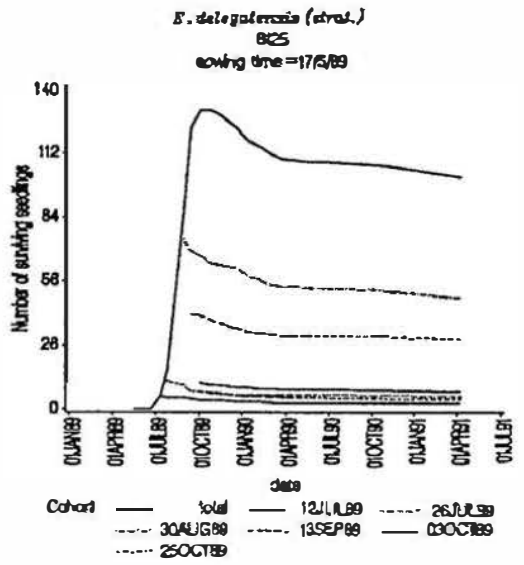

E. amygdalina
Bi25
sowing time = 13/11/89
Number of surviving seedlings
3.0
2.4
1.8
1.2
0.6
0.0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total

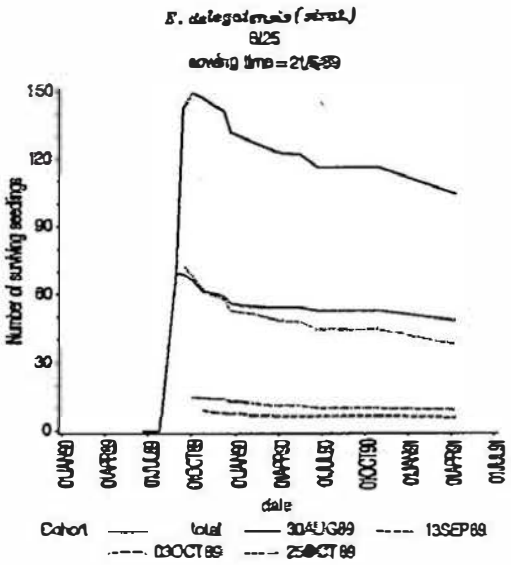

E. amygdalina
Bi25
sowing time = 3/10/89
Number of surviving seedlings
30
24
18
12
6
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
13NOV89
06DEC89
14MAY90

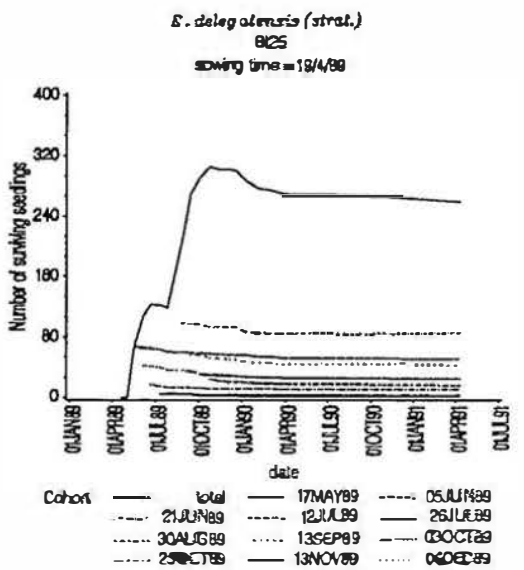

E. amygdalina
Bi25
sowing time = 6/12/89
Number of surviving seedlings
2.0
1.6
1.2
0.8
0.4
0.0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total

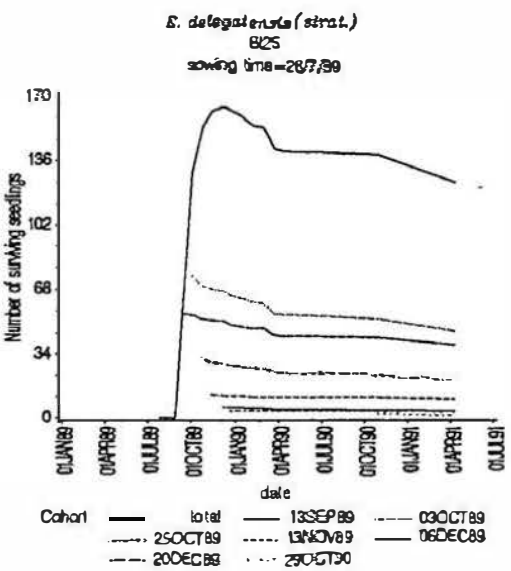

E. amygdalina
Bi25
sowing time = 30/8/89
Number of surviving seedlings
50
40
30
20
10
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
03OCT89
25OCT89
13NOV89
06DEC89

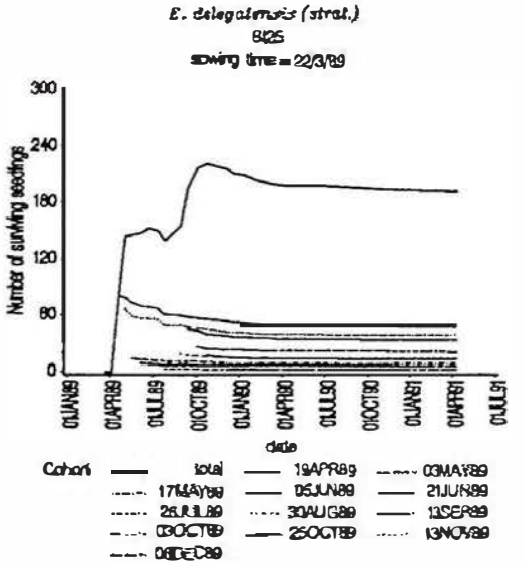

E. amygdalina
Bi25
sowing time = 6/2/90
Number of surviving seedlings
30
24
18
12
6
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
14MAY90
25OCT90

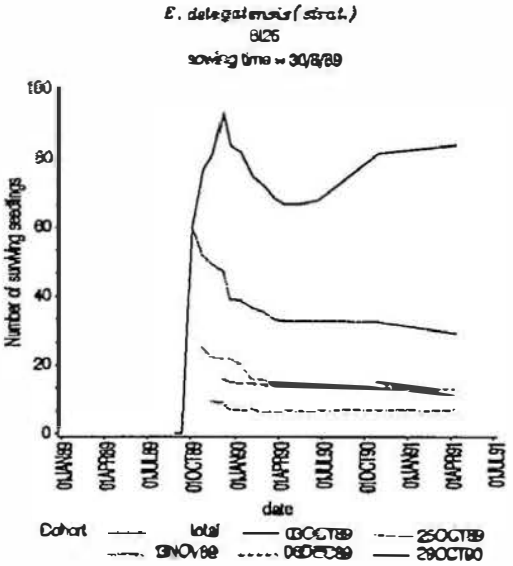

E. delegatensis (strat.)
8125
sowing time =17/5/89
Number of surviving seedlings
date
Cohort
total
12JUL89
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89

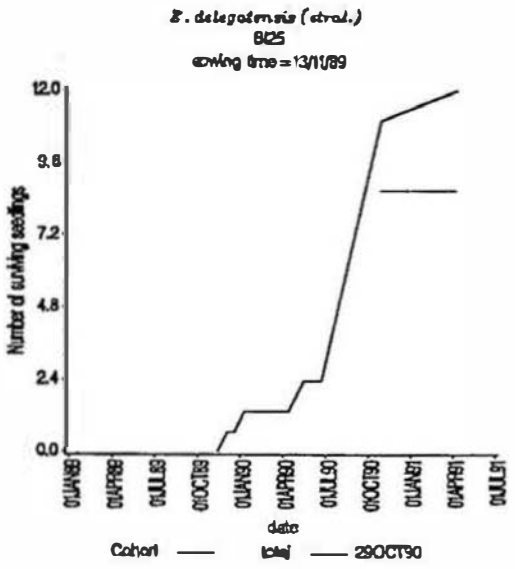

E. delegatensis (strat.)
8125
sowing time = 21/6/89
Number of surviving seedlings
date
Cohort
total
30AUG89
13SEP89
03OCT89
25OCT89

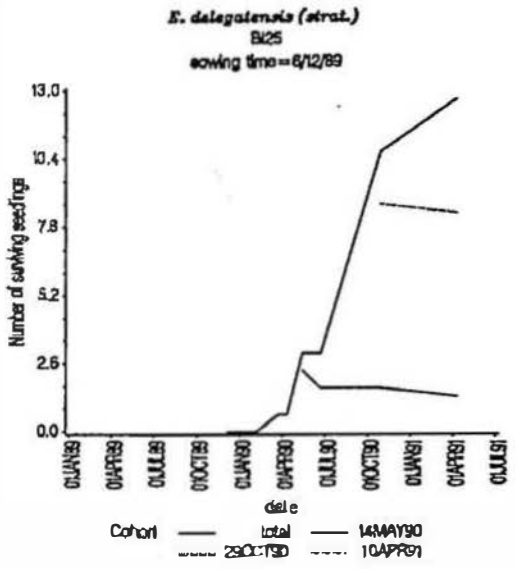

E. delegatensis (strat.)
8125
sowing time = 19/4/89
Number of surviving seedlings
date
Cohort
total
17MAY89
05JUN89
21JUN89
12JUL89
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

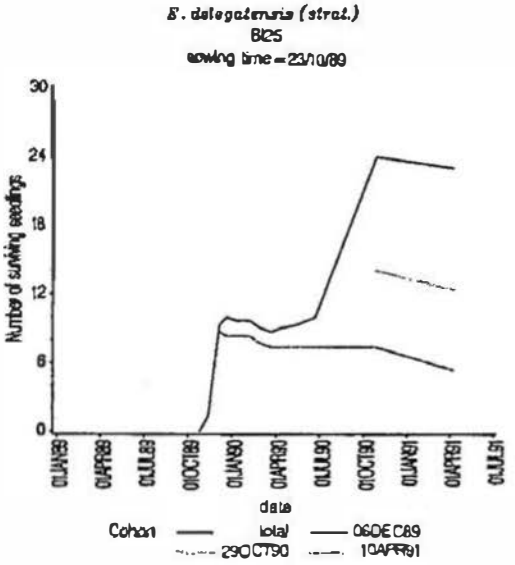

E. delegatensis (strat.)
8125
sowing time = 26/7/89
Number of surviving seedlings
date
Cohort
total
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89
20DEC89
29OCT90

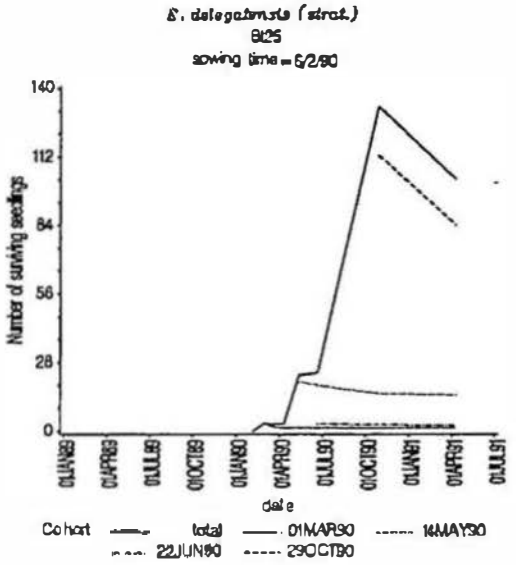

E. delegatensis (strat.)
8125
sowing time = 22/3/89
Number of surviving seedlings
date
Cohort
total
19APR89
03MAY89
17MAY89
05JUN89
21JUN89
26JUL89
30AUG89
13SEP89
03OCT89
25OCT89
13NOV89
06DEC89

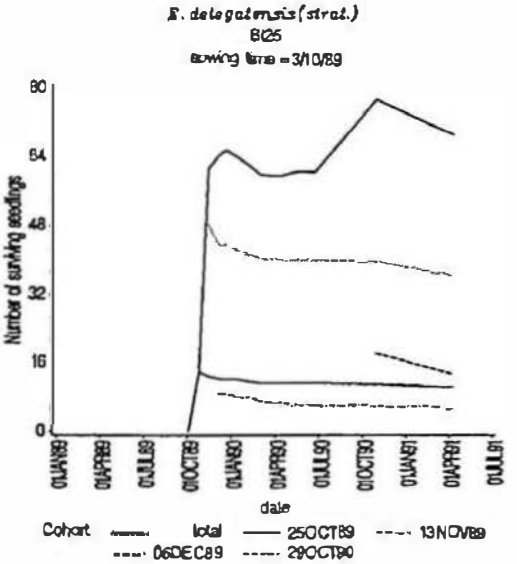

E. delegatensis (strat.)
8125
sowing time = 30/8/89
Number of surviving seedlings
date
Cohort
total
03OCT89
25OCT89
13NOV89
06DEC89
29OCT90


E. delegatensis (strat.)
B125
sowing time = 13/11/89
Number of surviving seedlings
12.0
9.6
7.2
4.8
2.4
0.0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
29OCT90


E. delegatensis (strat.)
B125
sowing time = 6/12/89
Number of surviving seedlings
13.0
10.4
7.8
5.2
2.6
0.0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
14MAY90
29OCT90
10APR91

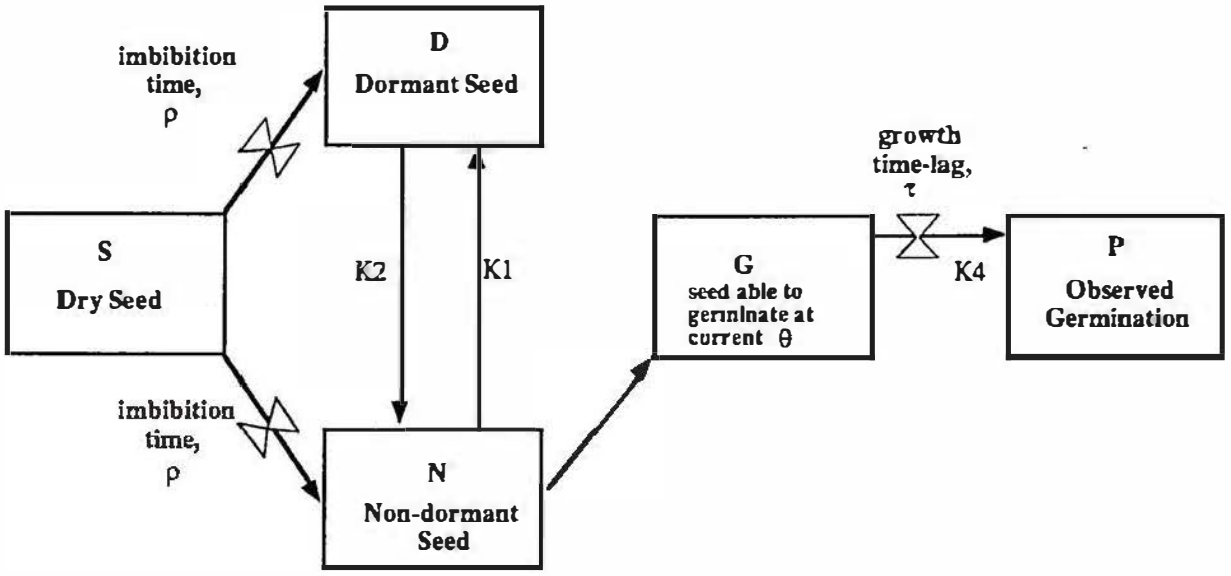

E. delegatensis (strat.)
B125
sowing time = 23/10/89
Number of surviving seedlings
30
24
18
12
6
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
06DEC89
29OCT90
10APR91


E. delegatensis (strat.)
B125
sowing time = 6/2/90
Number of surviving seedlings
140
112
84
56
28
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
01MAR90
14MAY90
22JUN90
29OCT90


E. delegatensis (strat.)
B125
sowing time = 3/10/89
Number of surviving seedlings
80
64
48
32
16
0
01JAN89
01APR89
01JUL89
01OCT89
01JAN90
01APR90
01JUL90
01OCT90
01JAN91
01APR91
01JUL91
date
Cohort
total
25OCT89
13NOV89
06DEC89
29OCT90

APPENDIX 4: Mathematical solution of differential equations associated with germination model.

These equations deal with the situation where all seeds in **G** are capable of progressing to **P** and the number of seeds in **G** and **P** is initial equal to zero. Adjustments for water stress situations (see Chapter 7) and for different initial starting conditions are trivial and would only serve to confuse the mathematical development. The following solution was provide by Dr. David Paget, Mathematics Department, University of Tasmania.



The system indicated above is modelled by the following set of equations:

$$D'(t) = -k2.D(t)+k1.N(t) \quad (1)$$

$$N'(t) = k2.D(t)-(k1+k3).N(t) \quad (2)$$

$$G(t) = k3.N(t)-k4.G(t-\tau) \quad (3)$$

$$P'(t) = k4.G(t-\tau) \quad (4)$$

With initial conditions at $t=\rho$:

$$D(\rho) = D_o \quad (5)$$

$$N(\rho) = No \quad (6)$$

$$G(\rho) = 0 \quad (7)$$

$$P(\rho) = 0 \quad (8)$$

The rates *k1, k2, k3* are activated after time t =ρ.

The rate *k4* is activated after time t =ρ+τ.

For t≥ρ , from (1) and (2)

$$N''(t)+2pN'(t)+qN(t)=0 \quad (9)$$

where $k1+k2+k3=2p$ and $k2.k3=q$ (10)

If $p^2-q=k^2>0$ then

$$N(t)=Ae^{-\alpha t}+Be^{-\beta t} \quad (11)$$

where $\alpha=p+k$ and $\beta=p-k$ (12)

from (2) and (11)

$$D(t)=\frac{1}{k2}\left((k1+k3-\alpha)Ae^{-\alpha t}+(k1+k3-\beta)Be^{-\beta t}\right) \quad (13)$$

from (6) and (11)

$$Ae^{-\alpha\rho}+Be^{-\beta\rho}=N_o \quad (14)$$

from (5) and (13)

$$(k1+k3-\alpha)Ae^{-\alpha\rho}+(k1+k3-\beta)Be^{-\beta\rho}=k2(1-N_o) \quad (15)$$

Solving (14) and (15) for A and B:

$$A=\frac{k3N_o-\beta}{2k}, \quad B=\frac{\alpha-k3N_o}{2k} \quad (16)$$

Thus for all t≥ρ,

$$N(t)=\frac{\alpha A}{k3}.e^{-\alpha t}+\frac{\beta B}{k3}.e^{-\beta t} \quad (17)$$

$$D(t)=(1-\frac{\alpha}{k3}).A.e^{-\alpha t}+(1-\frac{\beta}{k3}).B.e^{-\beta t} \quad (18)$$

Now to evaluate G(t) there are several stages:

stage (i) ρ≤t≤ρ+τ, stage (ii) ρ+τ≤t≤ρ+2τ, stage (iii) ρ+2τ≤t≤ρ+3τ .....

For stage (i) we have from (3) and (7)

$$G'(t)=k3N(t) \text{ with } G(\rho)=0 \quad (19)$$

$$\therefore G(t)=\int_\rho^t G'(t).dt=\int_\rho^t k3.N(t).dt$$

Thus from (17) we have, for ρ≤t≤ρ+τ,

$$G(t)=1-\alpha A.e^{-\alpha t}+\beta B.e^{-\beta t} \quad (20)$$

For stage (ii) from (3)

$$G'(t)=k3.N(t)-k4.G(t-\tau) \quad (21)$$

But ρ≤t-τ≤ρ+τ ∴ G(t-τ) is given by (20)

$$G'(t)=\alpha A.e^{-\alpha t}+\beta B.e^{-\beta t}-k4(1-\alpha A.e^{-\alpha(t-\tau)}+\beta B.e^{-\beta(t-\tau)}) \quad (22)$$

$$\therefore G(t)=1-k4(t-\tau-\frac{A}{\alpha}-\frac{B}{\beta})-A.(\frac{k4}{\alpha}+e^{-\alpha\tau}).e^{-\alpha(t-\tau-\rho)}-A.(\frac{k4}{\beta}+e^{-\beta\tau}).e^{-\beta(t-\tau-\rho)} \quad (23)$$

In general it can be shown by induction that for $(m-1)\tau \le t \le m\tau$:

$$G(t)=X_{m-1}(t)-A\frac{r^m-e^{-m\alpha\tau}}{r-e^{-\alpha\tau}}e^{-\alpha(t-\rho-(m-1)\tau)}-B\frac{s^m-e^{-m\beta\tau}}{s-e^{-\beta\tau}}e^{-\beta(t-\rho-(m-1)\tau)} \qquad (24)$$

where:

$r=\frac{k4}{\alpha}$, $s=\frac{k4}{\beta}$, $X_o(t)=1$

and $X_{m-1}(t)$ is a polynomial of degree m-1 given recursively by:

$$X_i(t)=X_{i-1}(\rho+i.\tau)+A.r^i+B.s^i - k4\int_{\rho+(i-1)\tau}^{t-\tau} X_{i-1}(u).\,du. \qquad (25)$$

To calculate the polynomials:

Suppose $X_{n-1}(t)=\sum_{i=0}^{n-1} a_{i,n-1}(t-\rho-(n-1)\tau)^i$

$$\begin{aligned}
X_n(t) &= X_{n-1}(\rho+n\tau)-k4\int_0^{t-\rho-n\tau} Xn\text{-}1(v-\rho-(n-1)\tau).dv-Br^n-Cs^n \\
&= \sum_{i=0}^{n-1} a_{i,n-1}\tau^i - k4\sum_{i=0}^{n-1} a_{i,n-1}\int_0^{t-\rho-n\tau} v^i dv-Br^n-Cs^n \\
&= \sum_{i=0}^{n-1} a_{i,n-1}\tau^i -Br^n-Cs^n -k4\sum_{i=0}^{n-1}\left(\frac{a_{i,n-1}}{i+1}(t+\rho+n\tau)^{i+1}\right) \\
&= \sum_{i=0}^{n-1} a_{i,n-1}\tau^i -Br^n-Cs^n -k4\sum_{i=1}^{n}\left(\frac{a_{i,n-1}}{i}(t+\rho+n\tau)^{i}\right) \\
&= \sum_{i=0}^{n} a_{i,n}(t+\rho+n\tau)^i
\end{aligned}$$

$$\therefore \quad a_{0,n}=\sum_{i=0}^{n-1} a_{i,n-1}\tau^i-Br^n-Cs^n$$

and $a_{i,n}=-\frac{k4}{i}i=0,n-1,)a_{i-1,n-1}$, $\quad i=1,2\ldots,n.$

Finally we note from (3) and (4) that

$$P'(t)+G'(t)=k3N(t)=N(t)=\alpha A.e^{-\alpha t}+\beta B.e^{-\beta t} \qquad (26)$$

so that

$P(t)+G(t)=1-A.e^{-\alpha t}-B.e^{-\beta t}$, for all t

and hence,

$$P(t)=1-A.e^{-\alpha t}-B.e^{-\beta}-G(t) \qquad (27)$$

APPENDIX 5: Listing of program used to calculate surface soil moisture

```
data weatset; set weather;
weather is a data set that contains the following variables:
datetime=the date and time expressed in seconds since 00:00 01/01/1960
ta=ambient temperature (°C)
RH=relative humidity (%)
C=cloud cover (proporiton)
U=wind speed(m/sec)
rain=accumulated rainfall since last recording (mm)
retain sveang(0) sveRH (91) svedtime (949532400) sveta (11)
      sveC (1) sveU (0) sveRg (0);
dtnow=datetime;
hour=hour(dtnow); day=datepart(dtnow); month=month(day);
elapsed=(dtnow-svedtime)/60;
the following lookup table calculates the sunangle (degrees) and global
irradiance (W/m2) for each hour of each day of the year.
 if month=1 then do;
 if hour= 5 then do; sunangle= 4 ; Rg= 4.14 ; end;
else if hour= 6 then do; sunangle= 15 ; Rg= 123 ; end;
else if hour= 7 then do; sunangle= 25 ; Rg= 322 ; end;
else if hour= 8 then do; sunangle= 37 ; Rg= 530 ; end;
else if hour= 9 then do; sunangle= 48 ; Rg= 716 ; end;
else if hour= 10 then do; sunangle= 58 ; Rg= 862 ; end;
else if hour= 11 then do; sunangle= 67 ; Rg= 955 ; end;
else if hour= 12 then do; sunangle= 71 ; Rg= 987 ; end;
else if hour= 13 then do; sunangle= 68 ; Rg= 955 ; end;
else if hour= 14 then do; sunangle= 59 ; Rg= 863 ; end;
else if hour= 14 then do; sunangle= 59 ; Rg= 863.03 ; end;
else if hour= 15 then do; sunangle= 49 ; Rg= 717.41 ; end;
else if hour= 16 then do; sunangle= 38 ; Rg= 531.18 ; end;
else if hour= 17 then do; sunangle= 27 ; Rg= 323.27 ; end;
else if hour= 18 then do; sunangle= 16 ; Rg= 124.44 ; end;
else if hour= 19 then do; sunangle= 5 ; Rg= 4.28 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=2 then do;
 if hour= 6 then do; sunangle= 9 ; Rg= 64.81 ; end;
else if hour= 7 then do; sunangle= 20 ; Rg= 252.21 ; end;
else if hour= 8 then do; sunangle= 31 ; Rg= 461.81 ; end;
else if hour= 9 then do; sunangle= 42 ; Rg= 652.49 ; end;
else if hour= 10 then do; sunangle= 52 ; Rg= 802.51 ; end;
else if hour= 11 then do; sunangle= 61 ; Rg= 898.08 ; end;
else if hour= 12 then do; sunangle= 65 ; Rg= 931.15 ; end;
else if hour= 13 then do; sunangle= 63 ; Rg= 899.04 ; end;
else if hour= 14 then do; sunangle= 56 ; Rg= 804.35 ; end;
else if hour= 15 then do; sunangle= 47 ; Rg= 655.05 ; end;
else if hour= 16 then do; sunangle= 36 ; Rg= 464.84 ; end;
else if hour= 17 then do; sunangle= 25 ; Rg= 255.3 ; end;
else if hour= 18 then do; sunangle= 14 ; Rg= 67.04 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=3 then do;
 if hour= 6 then do; sunangle= 3 ; Rg= 5.46 ; end;
else if hour= 7 then do; sunangle= 14 ; Rg= 141.33 ; end;
```

```
else if hour= 8 then do; sunangle= 25 ; Rg= 341.64 ; end;
else if hour= 9 then do; sunangle= 36 ; Rg= 531.78 ; end;
else if hour= 10 then do; sunangle= 45 ; Rg= 683.04 ; end;
else if hour= 11 then do; sunangle= 52 ; Rg= 779.57 ; end;
else if hour= 12 then do; sunangle= 56 ; Rg= 812.63 ; end;
else if hour= 13 then do; sunangle= 54 ; Rg= 779.36 ; end;
else if hour= 14 then do; sunangle= 48 ; Rg= 682.63 ; end;
else if hour= 15 then do; sunangle= 40 ; Rg= 531.22 ; end;
else if hour= 16 then do; sunangle= 30 ; Rg= 340.99 ; end;
else if hour= 17 then do; sunangle= 19 ; Rg= 140.72 ; end;
else if hour= 18 then do; sunangle= 8 ; Rg= 5.31 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=4 then do;
 if hour= 7 then do; sunangle= 7 ; Rg= 24.62 ; end;
else if hour= 8 then do; sunangle= 18 ; Rg= 177.04 ; end;
else if hour= 9 then do; sunangle= 28 ; Rg= 351.67 ; end;
else if hour= 10 then do; sunangle= 36 ; Rg= 496.3 ; end;
else if hour= 11 then do; sunangle= 41 ; Rg= 590.01 ; end;
else if hour= 12 then do; sunangle= 44 ; Rg= 622.59 ; end;
else if hour= 13 then do; sunangle= 42 ; Rg= 590.84 ; end;
else if hour= 14 then do; sunangle= 37 ; Rg= 497.88 ; end;
else if hour= 15 then do; sunangle= 29 ; Rg= 353.82 ; end;
else if hour= 16 then do; sunangle= 19 ; Rg= 179.36 ; end;
else if hour= 17 then do; sunangle= 9 ; Rg= 25.95 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=5 then do;
 if hour= 8 then do; sunangle= 11 ; Rg= 53.2 ; end;
else if hour= 9 then do; sunangle= 20 ; Rg= 190.66 ; end;
else if hour= 10 then do; sunangle= 27 ; Rg= 318.29 ; end;
else if hour= 11 then do; sunangle= 32 ; Rg= 403.46 ; end;
else if hour= 12 then do; sunangle= 33 ; Rg= 433.29 ; end;
else if hour= 13 then do; sunangle= 31 ; Rg= 404.05 ; end;
else if hour= 14 then do; sunangle= 26 ; Rg= 319.39 ; end;
else if hour= 15 then do; sunangle= 19 ; Rg= 192.07 ; end;
else if hour= 16 then do; sunangle= 10 ; Rg= 54.4 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=6 then do;
 if hour= 8 then do; sunangle= 6 ; Rg= 5.77 ; end;
else if hour= 9 then do; sunangle= 14 ; Rg= 92.96 ; end;
else if hour= 10 then do; sunangle= 20 ; Rg= 199.93 ; end;
else if hour= 11 then do; sunangle=25 ; Rg= 275.19 ; end;
else if hour= 12 then do; sunangle= 26 ; Rg= 301.75 ; end;
else if hour= 13 then do; sunangle= 24 ; Rg= 275.12 ; end;
else if hour= 14 then do; sunangle= 20 ; Rg= 199.81 ; end;
else if hour= 15 then do; sunangle= 13 ; Rg= 92.82 ; end;
else if hour= 16 then do; sunangle= 5 ; Rg= 5.72 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=7 then do;
 if hour= 8 then do; sunangle= 4 ; Rg= 2.73 ; end;
else if hour= 9 then do; sunangle= 12 ; Rg= 78.85 ; end;
else if hour= 10 then do; sunangle= 19 ; Rg= 180.91 ; end;
else if hour= 11 then do; sunangle= 23 ; Rg= 253.66 ; end;
else if hour= 12 then do; sunangle= 25 ; Rg= 279.19 ; end;
else if hour= 13 then do; sunangle= 23 ; Rg= 252.88 ; end;
else if hour= 14 then do; sunangle= 19 ; Rg= 179.51 ; end;
else if hour= 15 then do; sunangle= 13 ; Rg= 77.27 ; end;
else if hour= 16 then do; sunangle= 5 ; Rg= 2.37 ; end;
else do; sunangle=0; Rg=0; end; end;
```

```
 else if month=8 then do;
 if hour= 8 then do; sunangle= 7 ; Rg= 26.17 ; end;
else if hour= 9 then do; sunangle= 16 ; Rg= 143.31 ; end;
else if hour= 10 then do; sunangle= 23 ; Rg= 261.57 ; end;
else if hour= 11 then do; sunangle= 28 ; Rg= 341.72 ; end;
else if hour= 12 then do; sunangle= 30 ; Rg= 369.57 ; end;
else if hour= 13 then do; sunangle= 29 ; Rg= 341.19 ; end;
else if hour= 14 then do; sunangle= 24 ; Rg= 260.58 ; end;
else if hour= 15 then do; sunangle= 18 ; Rg= 142.08 ; end;
else if hour= 16 then do; sunangle= 9 ; Rg= 25.35 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=9 then do;
 if hour= 7 then do; sunangle= 5 ; Rg= 5.37 ; end;
else if hour= 8 then do; sunangle= 16 ; Rg= 124.1 ; end;
else if hour= 9 then do; sunangle= 25 ; Rg= 285.95 ; end;
else if hour= 10 then do; sunangle= 32 ; Rg= 423.53 ; end;
else if hour= 11 then do; sunangle= 38 ; Rg= 513.13 ; end;
else if hour= 12 then do; sunangle= 39 ; Rg= 543.96 ; end;
else if hour= 13 then do; sunangle= 38 ; Rg= 512.74 ; end;
else if hour= 14 then do; sunangle= 33 ; Rg= 422.8 ; end;
else if hour= 15 then do; sunangle= 25 ; Rg= 284.96 ; end;
else if hour= 16 then do; sunangle= 16 ; Rg= 123.1 ; end;
else if hour= 17 then do; sunangle= 5 ; Rg= 5.09 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=10 then do;
 if hour= 6 then do; sunangle= 4 ; Rg= 0.02 ; end;
else if hour= 7 then do; sunangle= 15 ; Rg= 88.03 ; end;
else if hour= 8 then do; sunangle= 26 ; Rg= 274.03 ; end;
else if hour= 9 then do; sunangle= 36 ; Rg= 458.33 ; end;
else if hour= 10 then do; sunangle= 44 ; Rg= 606.46 ; end;
else if hour= 11 then do; sunangle= 49 ; Rg= 701.31 ; end;
else if hour= 12 then do; sunangle= 51 ; Rg= 733.75 ; end;
else if hour= 13 then do; sunangle= 48 ; Rg= 700.89 ; end;
else if hour= 14 then do; sunangle= 41 ; Rg= 605.67 ; end;
else if hour= 15 then do; sunangle= 32 ; Rg= 457.23 ; end;
else if hour= 16 then do; sunangle= 22 ; Rg= 272.78 ; end;
else if hour= 17 then do; sunangle= 11 ; Rg= 86.97 ; end;
else if hour= 18 then do; sunangle= 0 ; Rg= 0.01 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=11 then do;
 if hour= 6 then do; sunangle= 13 ; Rg= 41.51 ; end;
else if hour= 7 then do; sunangle= 24 ; Rg= 217.68 ; end;
else if hour= 8 then do; sunangle= 35 ; Rg= 424.38 ; end;
else if hour= 9 then do; sunangle= 45 ; Rg= 613.91 ; end;
else if hour= 10 then do; sunangle= 54 ; Rg= 763.14 ; end;
else if hour= 11 then do; sunangle= 61 ; Rg= 857.89 ; end;
else if hour= 12 then do; sunangle= 62 ; Rg= 890.06 ; end;
else if hour= 13 then do; sunangle= 58 ; Rg= 856.98 ; end;
else if hour= 14 then do; sunangle= 50 ; Rg= 761.38 ; end;
else if hour= 15 then do; sunangle= 40 ; Rg= 611.47 ; end;
else if hour= 16 then do; sunangle= 29 ; Rg= 421.51 ; end;
else if hour= 17 then do; sunangle= 18 ; Rg= 214.8 ; end;
else if hour= 18 then do; sunangle= 7 ; Rg= 39.72 ; end;
else do; sunangle=0; Rg=0; end; end;
  else if month=12 then do;
 if hour= 5 then do; sunangle= 6 ; Rg= 1.7 ; end;
else if hour= 6 then do; sunangle= 16 ; Rg= 110.7 ; end;
else if hour= 7 then do; sunangle= 27 ; Rg= 307.46 ; end;
```

```
else if hour= 8 then do; sunangle= 39 ; Rg= 515.38 ; end;
else if hour= 9 then do; sunangle= 49 ; Rg= 702.07 ; end;
else if hour= 10 then do; sunangle= 60 ; Rg= 848.13 ; end;
else if hour= 11 then do; sunangle= 67 ; Rg= 940.72 ; end;
else if hour= 12 then do; sunangle= 70 ; Rg= 972.24 ; end;
else if hour= 13 then do; sunangle= 65 ; Rg= 940.18 ; end;
else if hour= 14 then do; sunangle= 56 ; Rg= 847.1 ; end;
else if hour= 15 then do; sunangle= 45 ; Rg= 700.63 ; end;
else if hour= 16 then do; sunangle= 34 ; Rg= 513.66 ; end;
else if hour= 17 then do; sunangle= 23 ; Rg= 305.66 ; end;
else if hour= 18 then do; sunangle= 12 ; Rg= 109.22 ; end;
else if hour= 19 then do; sunangle= 2 ; Rg= 1.53 ; end;
else do; sunangle=0; Rg=0; end; end;
else do; sunagle=0; Rg=0; end;
the recordings are interpolated to give minutely readings
inRH=RH-sveRH; inta=ta-sveta; inC=clbi-sveC;
inRg=Rg-sveRg; inU=U-sveU; inRAIN=rain; insunang=sunangle-sveang;
 do i=1 to elapsed;
 datetime=svedtime+i*60;
 RH=sveRH+i*inRH/elapsed;
 Rg=sveRg+i*inRg/elapsed;
 Ta=sveTa+i*inTa/elapsed;
 C=sveC+i*inC/elapsed;
 U=sveU+i*inU/elapsed;
 sunangle=sveang+i*insunang/elapsed;
 RAIN=inrain/elapsed;
 output;
end;
sveRH=RH; svedtime=dtnow; sveang=sunangle;
sveRg=Rg; sveta=ta;
sveC=clbi; sveU=U;
keep datetime elapsed Rg RH Ta C U RAIN sunangle;
 run;
Data soilprof printset;
set weatset;
the initial soil profile conditions of temperature (TEMP), volumetric water
content (VVW), and water potential (pot) are set.
retain TEMP5(288) TEMP4(285) TEMP3(279) TEMP2(278) TEMP1(277)
      TEMP10(288) TEMP9(288) TEMP8(288) TEMP7(288) TEMP6(288)
      VVW5(0.22) VVW4(0.22) VVW3(0.22) VVW2(0.22) VVW1(0.22)
      VVW10(0.22) VVW9(0.22) VVW8(0.22) VVW7(0.22) VVW6(0.22)
      pot1(-10) pot2(-10) pot3(-10) pot4(-10) pot5(-10)
      pot6(-10) pot7(-10) pot8(-10) pot9(-10) pot10(-10)
      cumtime(0);
delt=60;
The actual solar radiation to reach the surface is calculate using the algorithm of
Nunez (1983)
if Rg>0 then do;
  m=cos(sunangle)+0.15*(93.885-sunangle**-1.253);
  t=1/Rg*exp(-0.112*m);
  psi=1-(1-t)*C;
  Rs=Rg*psi; end;
else do; Rs=0; end;
Temperature is coverted to kelvin
Ta=Ta+273;
because boundary layer resistances are unrealistically affected by very low wind
speeds, windspeeds below 1 m/sec are set to 1 m/sec.
if U<1 then U=1;
```

```
array TCOM(10) TCOM1-TCOM10; soil layer thickness (m)
array TEMP(10) TEMP1-TEMP10; soil layer temperature (K)
array VVW(10) VVW1-VVW10; soil layer volumetric water content (kg/kg)
array SPECH(10) SPECH1-SPECH10; soil layer specific heat (J/kg/K)
array COND(10) COND1-COND10; soil thermal conductivity (W/m/K)
array DENS(10) DENS1-DENS10; soil bulk density (kg/m3)
array VHCAP(10) VHCAP1-VHCAP10; volumetric heat capacity (W/m3/K)
array VHTC(10) VHTC1-VHTC10; thermal conductivity with respect to the
vapour phase
array FLOWT(10) FLOWT1-FLOWT10;
array NFLOWT(10) NFLOWT1-NFLOWT10;
array POT(10) POT1-POT10; water potential of soil layer (j/kg)
array HCWP(10) HCWP1-HCWP10; hydraulic conductivity with regards to the
water phase
array VP(10) VP1-VP10; vapour pressure
array HCT(10) HCT1-HCT10; hydraulic conductivity with regards to the water
phase
array FLOWW(10) FLOWW1-FLOWW10;
array NFLOWW(10) NFLOWW1-NFLOWW10;
array DEFIC(10) DEFIC1-DEFIC10;
the thickness of each soil layer to be examined is set (m)
TCOM1=0.01; TCOM2=0.01; TCOM3=0.02; TCOM4=0.02; TCOM5=0.02;
TCOM6=0.02; TCOM7=0.02; TCOM8=0.02; TCOM9=0.05; TCOM10=0.05;
do i=1 to 10;
  SPECH(i)=1000*(0.9513+0.7540*VVW(i))**2;
  COND(i)=0.25+2.134*VVW(i)**0.51612;
  DENS(i)=(1.6+VVW(i))*1000;
  VHCAP(i)=DENS(i)*SPECH(i);
  VHTC(i)=TEMP(i)*TCOM(i)*VHCAP(i);
end;
Es=0.95 (effective atmospheric emmissivity); Zo=0.005 (aerodynamic roughness
length of surface); a=0.16 (albedo); K=0.4; (von Karman constant) g=9.80665
(gravitational acceleration); SB=5.6697*10**-8 (Stephan Boltzman
constant);Da=1.2 (density of air); Cpa=1.01 (specific heat of air); b=0.05 ; z=0.05
(measurement height of ta);


calculate longwave radiation
Ea=1-0.261*exp(-0.000777*(Ta-273.2)**2);
Eac=Ea+C*(1-Ea)*(1-8/Ta);
Rl=Eac*SB*Ta**4;

calculate sensible heat flux density

Ri=-g*(TEMP1-Ta)*log(z/Zo)*z/(Ta*U**2);
IfRi<0 then E=Ri;
else E=Ri/(1-4.7*Ri);
ifE<0 then do;  x=(1-16*E)**0.25;
            Yh=2*log((1+x**2)/2);
            Ym=2*log((1+x)/2)+log((1+x**2)/2)-2*atan(x)+3.14159/2;
            end;
else do; Yh=-4.7*E; Ym=Yh; end;
ustar=K*U/(log(z/zo)-Ym);
H=Da*Cpa*K*ustar*(TEMP1-Ta)/(log(z/zo)-Yh);

calculate latent heat flux density

LE=b*Rs;
```

```
Hence find the sensible heat flux between soil and the atmosphere

FLOWT1=-(H+LE-Rs*(1-a)-Es*(RI-SB*TEMP1**4));

do i=2 to 10;
  FLOWT(i)=(TEMP(i-1)-TEMP(i))*COND(i)/TCOM(i);
end;
do i=1 to 9;
  NFLOWT(i)=FLOWT(i)-FLOWT(i+1);
end;
do i=1 to 10;
  VHTC(i)=VHTC(i)+NFLOWT(i)*DELT;
  TEMP(i)=VHTC(i)/(VHCAP(i)*TCOM(i));
end;
TEMP10=288;
R=461.5;  /*joules per kilogram per Kelvin */
do i=1 to 10;
 POR=0.4; soil porosity
claculate the soil torturosity
 TORT=0.3-0.77*VVW(i);  /* dimensionless */
 HUMID=(EXP((LOG(VVW(i))+2.43)/-2.27)+1)**-0.625;  calculate the
humidity of the air in soil
 ESAT=exp(21.814-5485.29/TEMP(i));  sat. vap press soil mbar
 ESOIL=ESAT*humid;                  vapour density of soil
 VP(i)=0.217*ESOIL/TEMP(i);         vapour concentration kg m-3
 KVW=4*10**-11*exp(24*VVW(i));
 SPVA=1.323*exp(17.27*(TEMP(i)-273)/(TEMP(i)-35.7))/TEMP(i);
 Dva=2.12*10**-5*(1+0.007*TEMP(i));
 HCWPV=-TORT*POR*DVA*SPVA*humid*18/(R*TEMP(i));
 HCWP(i)=KVW+HCWPV;
 HCT(i)=TORT*POR*DVA*HUMID*SPVA*(5307/TEMP(i)-1)/TEMP(i);
end;
/* calculate evaporation from the surface using Fickian Laws*/
 BLR=10*(log(0.05/0.005)-Yh)**2/(0.16*U);  boundary layer resisitance sec m-
1
 ESATAIR=exp(21.814-5485.29/TA);      saturated vapour pressure of air
mbar
 EAIR=ESATAIR*RH/100;                 vapour density of air
 VPA=0.217*EAIR/TA;                   vapour concentration kg m-3
 Ho=0.217*exp(21.814-5485.29/(TEMP1/2+TA/2))/TEMP1;
 HS=Ho*exp(POT1/(469.7*TEMP1));
Calculate evaporation and distribute rainfall through soil profile, starting at top
soil layer
 if rain=0 then
  EVAPO=(VPA-HS)/BLR;  evaporation downward flux +ve kg m-2 sec-1 =mm
sec-1
 else do;
  EVAPO=0;
  remrain=rain;
  do i=1 to 9;
   defic(i)=352*tcom(i)-vvw(i)*1600*tcom(i);
   if defic(i)<0 then defic(i)=0;
   if remrain>defic(i) then do;
      vvw(i)=0.22; remrain=remrain-defic(i); end;
   else if 0<remrain<=defic(i) then do;
      vvw(i)=vvw(i)+remrain/(1600*tcom(i)); remrain=0; end;
   else vvw(i)=vvw(i);
```

```
  end;
 end;
 FLOWW1=(VVW1*1600*TCOM1+evapo)/(1600*TCOM1)-VVW1 /* change
VVW in 1 sec */;
 do i=2 to 10;
   FLOWW(i)=(HCWP(i)*(pot(i-1)-pot(i))/10
       +TCOM(i)*HCT(i)*(TEMP(i-1)-TEMP(i)))/TCOM(i);
   end;
 do i=1 to 9;
     NFLOWW(i)=(FLOWW(i)-FLOWW(i+1));
     VVW(i)=VVW(i)+NFLOWW(i)*DELT;
     POT(i)=-exp(11.275-40.179*VVW(i)); /* J Kg-1 */
     end;
VVW10=0.22;  POT10=-10;
keep datetime pot1-pot10 vvw1-vvw10 temp1-temp10 ta rh c u rg sunangle rs
rain;
if mod(datetime,3600)=0 then output printset;
run;
```

APPENDIX 6: Program to predict field emergence of *E. delegatensis* seed.

Set the weather parameters and calculate the daily seed loss rate from the ground seed store (seasfac). ta=temperature °C, pot1=water potential of surface soil MPa, pot2=water potential of soil 2 cm below the surface MPa, pot3=water potential of soil 6 cm below the surface MPa

```
data dset; set w.msoil; if datetime>942537600;
temp=ta-273.2; pot1=pot1/1000; pot2=pot3/1000; pot3=pot5/1000;
if month(date)=1 then seasfac=0.01;
if month(date)=2 then seasfac=0.01;
if month(date)=3 then seasfac=0.01;
if month(date)=4 then seasfac=0.01;
if month(date)=5 then seasfac=0.01;
if month(date)=6 then seasfac=0.0075;
if month(date)=7 then seasfac=0.005;
if month(date)=8 then seasfac=0.005;
if month(date)=9 then seasfac=0.005;
if month(date)=10 then seasfac=0.0075;
if month(date)=11 then seasfac=0.01;
if month(date)=12 then seasfac=0.01;
end;
keep date datetime temp pot1 pot2 pot3 seasfac;

data dset; set w.bsoil; if datetime>942537600;
temp=ta-273.2; pot1=pot1/1000; pot2=pot3/1000; pot3=pot5/1000;
if month(date)=1 then seasfac=0.015;
if month(date)=2 then seasfac=0.01;
if month(date)=3 then seasfac=0.0075;
if month(date)=4 then seasfac=0.005;
if month(date)=5 then seasfac=0.0075;
if month(date)=6 then seasfac=0.01;
if month(date)=7 then seasfac=0.0115;
if month(date)=8 then seasfac=0.0125;
if month(date)=9 then seasfac=0.015;
if month(date)=10 then seasfac=0.0175;
if month(date)=11 then seasfac=0.02;
if month(date)=12 then seasfac=0.02;
keep date datetime temp pot1 pot2 pot3 seasfac;
```

The total seed population is divided into three populations those in depressions (DP), on hillocks (HL) & on the flat (FL)

```
data valuesDP;
retain svetherm 0 sveNo 0.66 sveDo 0.34 sveG 0 sveM 0
    svePo 0 svetot 0 svetime 942537600 sveRWC 0 C 0 svetau 10;
set dset;
PS=svePo; Ns=sveNo; Ss=sveDo; M=sveM;
```

calculate elapsed time and current time and convert from seconds into days

```
time=(datetime-svetime)/86400;
tottime=svetot+time;
```

calculate parameter values from temperature and water potential

```
if temp gt 15 then k1=6.24*exp(-108.50/temp);
else k1=0.59-0.04*temp+6.24*exp(-108.50/temp);
if temp le 5 then k2=0.80277;else k2=0.00001;
k3=0.10*exp(3.78*(1/20.76-1/temp)*(1+0.002)/
  (1+0.002*exp(1904*(1/20.76-1/temp)));
if pot3<-0.25 then do;
k4=exp(2.75*pot3)*(0.22*exp(88.88*(1/20.29-1/temp)*2.42)/
  (1+1.42*exp(151.64*(1/20.29-1/temp)))); end;
else do; k4=0.22*exp(88.88*(1/20.29-1/temp)*2.42)/
  (1+1.42*exp(151.64*(1/20.29-1/temp))); end;
```

calculate rho & RWC at end of time interval

```
SOIL=0.44*exp(0.20*pot3);
if pot3<-2 then do; if soil<sveRWC then sveRWC=soil; svetherm=0;
savetau=10; end;
if sveRWC>=0.40 then do; rho=0; RWC=0.4; end;
else do;
if pot3<-0.5 then do;
RWC=sveRWC+(0.44-sveRWC)*(0.03*temp)*exp(3.00*(pot3+0.5))*time*24
   /(1+exp(3*(pot3+0.5))*0.03*temp*time*24);
rho=(0.4/((0.44-0.4)*(0.03*temp)*exp(3.00*(pot3+0.5)))-sverwc/((0.44-
sverwc)*0.03*temp))/24;; end;
else do;
RWC=sveRWC+(0.44-sveRWC)*(0.03*temp)*time*24
   /(1+0.03*temp*time*24);
rho=(0.4/((0.44-0.4)*(0.03*temp)*exp(3.00*(pot3+0.5)))-sverwc/((0.44-
sverwc)*0.03*temp))/24;; end;
end;
if rho>10 then rho=10;
```

calculate proportion of tau remaining

```
if rho-time >= 0 then do; tau=10; thermal=0; end;
else do;
 if pot3>=-0.1 then do;
    if temp>2.89 then thermal=svetherm+(time-rho)*(temp-2.89);
     else thermal=svetherm;
    if svetherm < 118.29 then do;
     if temp>2.89 then tau=(118.29-svetherm)/(temp-2.89);
     else tau=svetau; end;
     else do; tau=0; end;
    if svetherm>118.29 then tau=0; if tau >10 then tau=10; end;
 else do;
    if temp>2.89 then
     thermal=svetherm+(time-rho)*(temp-2.89)/exp(-1.07*pot3);
    else thermal=svetherm;
    if svetherm < 118.29 then do;
     if temp>2.89 then
      tau=exp(-1.067*pot3)*(118.29-svetherm)/(temp-2.89);
      else tau=svetau; end;
     else do; tau=0;  end;
    if svetherm>118.29 then tau=0; if tau >10 then tau=10; end;
end;
```

calculate proportion that can germinate at current water potential

```
if pot3>=-0.075 then gamma=1; else gamma=exp(3.40*POT3);
if soil>0.1 then do; G=sveG; G1s=gamma*G; G2s=(1-gamma)*G; end;
else do; M=M+sveG; G=0; G1s=0; G2s=0; end;

Set up some aliases
po=(k1+k2+k3)/2;
q=k2*k3;
k=sqrt(po*po-q);
alpha=po+k;
beta=po-k;
A=(k3*Ns-beta*(1-G-ps-M))/(2*k);
B=(-k3*Ns+alpha*(1-G-ps-M))/(2*k);
C=gamma*(1-A/(exp(alpha*tau)-alpha/k4)
    -B/(exp(beta*tau)-beta/k4)-ps-M);

Calculate compartmental quantities at the end of the time interval
  N=alpha*A/k3*exp(-alpha*(time-rho))
   +(beta*B)/k3*exp(-beta*(time-rho));

  D=(-alpha/k3+1)*A*exp(-alpha*(time-rho))
   +(-beta/k3+1)*B*exp(-beta*(time-rho));

if time le rho then do; P1=ps;
N=Ns;
D=Ss;
G1=g1s; G2=g2s; G=G1+G2; M=M;
end;
else if time>=(tau+rho) then do;
  G2=(1-gamma)*(1-A*exp(-alpha*(time-rho))-B*exp(-beta*(time-rho))-ps-M);
  G1=(1/(k4/alpha*exp(alpha*tau)-1))*gamma*A*exp(-alpha*(time-rho))
  +(1/(k4/beta*exp(beta*tau)-1))*gamma*B*exp(-beta*(time-rho)
  +C*exp(-k4*(time-rho-tau));
  G=G1+G2;
  P1=1-G-D-N-M;
  end;
else do;
  G1=gamma*(1-A*exp(-alpha*(time-rho))-B*exp(-beta*(time-rho))-ps-M);
  G2=(1-gamma)*(1-A*exp(-alpha*(time-rho))-B*exp(-beta*(time-rho))-ps-M);
  G=G1+G2;
  P1=Ps;
  end;

Reset some some counters and apply daily seed loss rate, set contribution of this
microsite to total seed population, in this cas 34%
kill=1-seasfac*time; killS=1-seasfac*time;
svetherm=thermal; sveNo=killS*I; sveDo=killS*S; sveRWC=RWC;  svetau=tau;
sveG=kill*G; svePo=P1; svetot=tottime; svetime=datetime;
sveM=1-svePo-sveDo-sveNo-sveG;
NDP=N*0.334; DDP=D*0.334; G1DP=G1*0.334;
RWCDP=RWC; rhoDP=rho; tauDP=tau;
G2DP=G2*0.334; MDP=M*0.334; P1DP=P1*0.334;
if mod(datetime,86400)=0 then output;
keep date tottime temp pot3 RWCDP rhoDP tauDP NDP DDP G1DP G2DP
MDP P1DP;
run;

Repeat for other microsites DP and FL using appropriate soil moisture
conditions creating datasets valuesDP and valuesFL
```

Merge data sets and output results

```
data values1; merge valuesHL valuesDP valuesFL;
N=NHL+NDP+IFL; D=DHL+DDP+SFL; G1=G1HL+G1DP+G1FL;
G2=G2HL+G2DP+G2FL;
 M=MHL+MDP+MFL; PL=PLHL+PLDP+PLFL;
proc print data=values1;  var date temp pot1 pot2 pot3 rhoHL rhoFL
        rhoDP tauHL tauFL tauDP RWCHL RWCFL RWCDP N D G1 G2 Pl M;
format date date.; run;
```